TEMPORAL AND SPATIAL VARIABILITY OF SINKING PARTICLES IN THE SOUTHEAST BERING SEA

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A

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Abstract

The factors affecting the timing, quantity and fate of southeastern Bering Sea primary production, including coupling to higher trophic levels, were investigated via sinking particles collected using sediment traps. Stable carbon and nitrogen isotopic composition and lipid composition of zooplankton and sinking particles were measured for middle (M2) and outer (M3) shelf samples collected during 1997-2000. The quantity collected by the M2 sediment trap was high in late summer and fall, as well as during spring blooms, and was much greater than that collected at M3. M2 zooplankton and trap samples were enriched in ^{15}N and ^{13}C over those from M3. This could be explained by greater primary productivity over the middle shelf, associated with consumption of more of the available inorganic nitrogen.

M2 sediment trap samples contained more fatty acids typical of diatoms, while M3 samples contained more fatty acids typical of zooplankton. Diatoms were much more numerous in the M2 than the M3 trap. Cholesterol was the dominant sterol, indicating that much of the material in the traps was fecal matter. During two of the years sampled, 1997 and 1999, ice edge blooms occurred from late April to early May. Ice receded earlier in 1998 and 2000, so phytoplankton bloomed in open water in late May. Lipids indicating greater phytoplankton input were high in the M2 trap during the ice-edge bloom years. Conversely, in 1998 and 2000, there was greater coupling between phytoplankton and zooplankton, much of the material collected was fecal pellets rather than intact diatoms and lipids were more characteristic of zooplankton sources.

In zooplankton some monounsaturated fatty acids decreased sharply between February and April, reflecting mobilization of lipids for egg production. A polyunsaturated fatty acid characteristic of prymnesiophytes was elevated in winter and spring 1998 zooplankton, resulting from grazing of the 1997 coccolithophorid bloom.

Overall, the results indicate that primary productivity is greater at M2 than at M3. Much of the annual primary production occurs outside of the April-May spring bloom period. Grazing of primary production is greater at M3 than M2, and at M2 more primary production is grazed in warm than cold years.

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Chapter 1.

Introduction

1.1. Historical Perspective

The southeastern Bering Sea shelf has been the home of abundant marine life for millennia (Fay, 1981). Native peoples have relied upon its resources, including whales, seals, sea lions, sea otters and numerous fishes, for food, clothing, shelter and fuel. Abuse of the Bering Sea's resources over the last two-and-a-half centuries have decimated some species, such as the Steller sea cow, extirpated just 27 years after its discovery by Europeans. Sea otters, fur seals, walruses and bowhead whales were all threatened with extinction at times during the last two centuries. Most recently the Steller sea lion has been declared an Endangered Species, but the cause of its steep population decline is unknown (Paine *et al.*, 2003). The Bering Sea has undergone tremendous changes over the past four decades. Over-fishing and changing ocean conditions probably both contributed to the depletion of important fish and shellfish stocks, including those of salmon, king crab, Tanner crab, and herring (Wespestad and Gunderson, 1991; National Research Council, 1996; Kruse, 1998; Paine *et al.*, 2003).

1.2. Past Research on the Bering Sea Ecosystem

Past research efforts in the Bering Sea included the Outer Continental Shelf Environmental Assessment Program, OCSEAP, in which detailed hydrographic

surveys were done during the 1960s and 1970s. Processes and Resources of the Bering Sea (PROBES), conducted from the late 1970s to the early 1980s, studied the entire southeast Bering Sea ecosystem, from its physical, chemical and biological oceanography to its fisheries, marine mammals and birds (Coachman, 1986; McRoy *et al.*, 1986; Walsh and McRoy, 1986). Inner SHelf Transfer and Recycling (ISHTAR) discovered the importance of nutrient rich waters from the basin and continental slope, which are the source of nutrients to the shelf, particularly the highly productive regions of the northern Bering and Chukchi Seas (McRoy, 1993; Hansell *et al.*, 1993).

1.3. Physical and Biological Setting

The Bering Sea shelf (Figure 1.1) is the broadest continental shelf outside the Arctic (Coachman, 1986). Over 500 km wide, it is a flat, featureless plain with mean bottom slopes of less than 0.2×10^{-3}; however, two zones of significantly steeper bottom slopes were identified (Askren, 1972) as the cause of distinct physical regimes. These zones are centered at the 50 m and 100 m isobaths, and, together with density fronts, they divide the shelf into three domains of differing hydrography: the coastal domain, the middle shelf and the outer shelf. The middle domain and outer domain are the sites of this study. The middle domain is separated from the coastal or "inner" domain by the inner front (Schumacher *et al.*, 1979). On the seaward side, the middle shelf is separated from the outer shelf by the middle front (Coachman and Charnell, 1979). The shelf break front separates the outer shelf from the shelf break and slope water. These fronts are not fronts in the strictest sense, but are zones that are much

broader than the water is deep, in which the horizontal temperature and salinity gradients are large relative to other shelf areas (Coachman, 1986).

Tides in this region, which are the mixed, semidiurnal type, have a range of more than 1 m. Over the entire southeastern shelf, tidal currents are the dominant water motion (Coachman, 1986; Pearson *et al.*, 1981); based on long-term current meter records from Kinder and Schumacher (1981), tidal currents are responsible for more than 80% of the total kinetic energy. In addition to tidal currents, a northwestward mean flow of 5 cm s^{-1} and cross-shelf flows are significant over the outer shelf (Schumacher and Kinder, 1983).

The water mass of the central shelf is distinguished by several characteristics (Kinder and Schumacher, 1981; Coachman, 1986), the most important among them being a two-layered vertical structure present during most of the year. The top layer (10 m to 40 m) forms due to melting ice and a decrease in winds during the late winter or early spring, or solar insolation during spring and summer. The bottom layer, segregated from other water masses by density fronts, is cold and homogenous. Other features of central domain water include small horizontal temperature and salinity gradients within the domain, and injections of freshwater supplied mainly by ice melt and rain.

According to Coachman (1986), the outer shelf water mass is a mixture of water from the middle shelf and Bering Sea basin. It is less strongly stratified than the middle shelf water, due to greater tidal and advective energy. The mixing zone between the wind mixed surface and tidally mixed bottom layers exhibits vertical fine

structure originating in the middle shelf front. Coachman (1986) states that this zone is very important to the vertical and cross-shelf flux of materials, including nutrients.

Physics strongly influences biology over the Bering Sea shelf. Primary productivity has largely been observed at the ice edge during colder years. As sea ice recedes, an intense phytoplankton bloom ensues due to an increase in light and melt water stabilization of the water column (Niebauer *et al.*, 1995). Phytoplankton found on the shelf are mainly diatoms such as *Chaetoceros* spp. *Coscinodiscus* spp., *Rhizosolenia* spp., *Thalassiosira* spp. and *Melosira sucata* (Allen, 1927; Kawarada and Ohwada, 1957; Alexander and Cooney, 1979) and various dinoflagellates. Smaller calanoid copepods, eg., *Pseudocalanus* spp., *Acartia* spp. and *Calanus marshallae,* and the euphausiid *Thysanoessa raschi* are typical consumers over the middle shelf. Typical zooplankton found on the outer shelf include the calanoid copepods *Neocalanus cristatus*, *Neocalanus plumchrus*, *Metridia* spp. and the euphausiid *Thysanoessa inermis*. Chaetognaths, *Sagitta* spp., are found on both the middle and outer shelves, as are pteropods and Scyphozoan medusae.

1.4. Recent Findings

Numerous recent studies have focused on climactic changes and how they affect the Bering Sea ecosystem. In the late 1970s the Bering Sea experienced a "regime shift", in which the climate changed from one with predominantly cold winters and high ice-coverage to one with warmer winters and less sea ice. This regime shift was correlated with the Pacific Decadal Oscillation (PDO) (Niebauer, 1998; Overland

et al., 2002). The PDO is a long-term pattern of climate variability in the Pacific. Colder weather in the Bering Sea is associated with a negative PDO, and warmer weather with a positive PDO. The PDO was positive from the late 1970s through the late 1990s. The PDO can be persistently positive or negative for 20-30 years and has been related to marine ecosystem changes in the Pacific as well as in the Bering Sea. For example, Hare and Francis (1995) linked variations in Alaskan salmon production to changes in the PDO. Declines in Bering Sea herring and increases in pollock also appear to be correlated with the "regime shift" (Wespestad and Gunderson, 1993; Wespestad, 1993). An increase in jellyfish on the middle shelf possibly linked to climate change has been reported (Brodeur *et al.*, 1999). Bering Sea weather and climate are also correlated with other, larger scale patterns including El-Niño Southern Oscillation (Niebauer and Day, 1989) and the Arctic Oscillation (Overland *et al.*, 1999). El-Niño Southern Oscillation linked climate variability is short-term compared with the PDO, and usually lasts from 6 to 18 months. Associated with an El-Niño and with unusually warm sea surface temperatures in 1997, a massive coccolithophorid bloom covered much of the southeastern shelf area (Stockwell *et al.*, 2001). The bloom has persisted during the years since (Stabeno and Hunt, 2002). A high mortality of shearwaters was recorded and linked to the unusual conditions (Baduini *et al.*, 2001).

Much attention has been focused on spring conditions and their role in ecosystem productivity. The increase in light in early spring and the increase in temperature ignite a spring bloom. During cold years sea ice is present over the

southeastern Bering Sea shelf. Melt water stabilizes the water column as early as March, but more typically in April, leading to a bloom at the ice edge (Niebauer *et al.*, 1995). During warm years that lack sea ice, thermal stratification of the water and an open water bloom occur later, usually in May. Hunt *et al.* (2002) developed a new theory concerning the controls on production at high trophic levels. They proposed that food web dynamics over the shelf are explained by the Oscillating Control Hypothesis (OCH). This theory states that variability in climate drives changes in productivity. For instance, during cold years, phytoplankton production is not efficiently grazed by zooplankton, because there are few adults during spring ice-edge blooms, leading to a lack of food for larval fishes. During a persistently cold regime, this leads to bottom-up limitation of the productivity at higher trophic levels, including pollock. Conversely, during warm years zooplankton graze the open water bloom that occurs later in spring, their production increases and abundant food exists for higher trophic levels. Under persistently warm conditions, top-down control (predation) limits the production of juvenile pollock and other forage fishes.

1.5. The Use of Sediment Traps to Collect Sinking Particles

Sediment traps are sampling devices, usually attached to moorings, that collect sinking particles representing input from the phytoplankton, microzooplankton and macrozooplankton comprising the pelagic food web. The quantity and composition of this sinking organic material reflects food web processes, including primary production, grazing by zooplankton and microbial decomposition, and changes in these

processes over time. Hence, sediment traps can be used to test hypotheses concerning the controls on ecosystem productivity.

Which organisms are responsible for the majority of the particle flux from the euphotic zone? The answer clearly varies in different ecosystems and at different times. In some cases, intact phytoplankton sink out of the euphotic zone to depth. In others zooplankton fecal matter is the major contributors to sinking particle flux. Zooplankton transform particles by assimilating some compounds from ingested food and egesting others, often repackaging them into fecal pellets. Large particles such as fecal pellets, although only a small fraction of the total particulate matter in the ocean, are responsible for more than 90% of the flux of material from the surface ocean to the deep sea (Bishop *et al.*, 1977).

In the upper ocean, swimmers (zooplankton which enter traps actively rather than by passive sinking) often constitute most of the collected material, and are very difficult to quantitatively separate from other particles (Lee and Hedges, 1988). However, swimmer-excluding traps (Peterson *et al.*, 1993) were used in this study (Figure 1.2). These sediment traps were also designed to minimize trapping efficiency artifacts, that is, to neither over- nor under-collect particulate matter relative to the actual flux of sinking material (Peterson *et al.*, 1993).

1.6. Stable Isotopes and Lipids in Ecological Studies

Stable nitrogen and carbon isotopic data have been used to investigate processes such as photosynthesis, nutrient uptake, particle regeneration via bacteria and

zooplankton, particle removal from the euphotic zone by zooplankton repackaging, and higher trophic level dynamics. For example, Mingawa and Wada (1984) and Fry (1988) found stepwise enrichment of the $\delta^{15}N$ of organisms with trophic level in food webs. Altabet and Francois (1994) related $\delta^{15}N$ of deep sea sediments to nitrate utilization in overlying surface waters. Wu *et al.* (1997, 1998) found a seasonal variation in the $\delta^{15}N$ and $\delta^{13}C$ values of sinking particulate matter in the northeastern Pacific Ocean, related to nutrient uptake and regeneration. Villinski *et al.* (2000) observed seasonal patterns of $\delta^{13}C$ in SPOM from the Ross Sea that were mainly related to phytoplankton growth rate or species-specific fractionation. Laboratory research has demonstrated that $\delta^{13}C$ of phytoplankton is largely controlled by growth rates and cell size (Laws *et al.*, 1995; Popp *et al.*, 1998).

Lipids, such as fatty acids, fatty alcohols and sterols, offer an excellent means to investigate certain aspects of ecosystems. For example, lipids can be used to indicate the presence of certain species, such as marine dinoflagellates. They possess high concentrations of the polyunsaturated fatty acids (PUFA) 18:4ω3, 18:5ω3 and especially 22:6ω3, but they are low in the monounsaturated fatty acid 16:1ω7. They are also high in a sterol called dinosterol (Volkman *et al.*, 1998). These kinds of indicators, along with microscopic identification to confirm species presence, can help shed light on the onset of a bloom, the end of a bloom, and the dominant phytoplankton groups.

Other signals come from zooplankton. For instance, calanoid copepods biosynthesize 20:1ω9 and 22:1ω11 fatty alcohols (Sargent and Henderson, 1986). Finding those compounds in sediment trap samples may indicate direct input from the crustaceans via fecal pellets, or it could indicate fecal material from a copepod predator. Only certain omnivorous zooplankton contain high concentrations of phytol, an indication of detrital feeding. All of these organisms are usually high in essential fatty acids (PUFA), especially 20:5ω3 and 22:6ω3.

1.7. The Dissertation

This research was part of the National Oceanic and Atmospheric Administration's (NOAA) Southeast Bering Sea Carrying Capacity (SEBSCC) program. The aim of this program was been to augment understanding of the ecosystem, through long-term monitoring, in order to understand factors that affect pollock (*Theragra chalcogramma*) survival and productivity (Macklin *et al.*, 2002). Sediment traps offered a means to continuously collect detritus produced by the pelagic food web, which could yield information on a variety of ecosystem events and processes occurring when shipboard observations were not possible. SEBSCC provided for the monitoring of temperature, salinity, nutrients, chlorophyll, and zooplankton during the 1997-2002 period. Hence, the particles collected by sediment trap could be interpreted in light of biophysical conditions.

This dissertation research aimed to test the following hypotheses on geographic and inter-annual variations in the southeastern Bering Sea shelf ecosystem:

- Ice-edge blooms over the middle shelf, which are little-grazed, contribute a substantial fraction of total annual sinking particles, consisting largely of intact diatoms, if ice is present in early spring (cold years).
- Spring blooms over the outer shelf, which are grazed by copepods, also make a distinct contribution to the annual sinking particle flux, but the sinking material contains more copepod and euphausiid-derived organic matter.
- Summer sinking particles in both the outer domain and middle shelf domain consist mainly of fecal pellets and "marine snow", the latter consisting primarily of organic matter derived from zooplankton.
- One control on the quantity of sinking organic matter in the summer is the extent of nutrient supply to the photic zone by wind mixing.

Later, based on the initial two years of data, the following hypotheses were added:

- A substantial part of the annual sinking particle flux occurs in fall, related to a substantial fall bloom.
- Calcium carbonate and organic matter from coccolithophores will continue to be a substantial part of the summer and fall particle flux over the middle shelf.

Samples were collected using sediment traps moored at two sites, site M2 on the middle shelf and site M3 on the outer shelf (Figure 1.1). Zooplankton were collected in winter and spring plankton tows at stations surrounding the trap sites.

Results of the sediment trap and zooplankton studies are discussed in four chapters, which were written as manuscripts for publication. Chapter 2 focuses on the stable carbon and nitrogen isotopic composition of suspended particles, sinking particles and zooplankton collected from the two sites during 1997-1999, and how these relate to nutrient supply and productivity over the shelf. Chapter 3 addresses differences between sinking particle fatty acids and neutral lipids collected at the two sites from February 1998 through January 2000, which reflect geographic and interannual differences in zooplankton grazing on phytoplankton. Chapter 4 examines the interannual variablity in sinking particulate matter at one site, M2, and specifically discusses how climactic changes affect the timing of the phytoplankton bloom and zooplankton grazing of the bloom. Chapter 5 focuses on major zooplankton lipids, fatty acids and fatty alcohols, and how they change seasonally and interannually at the two sites. The study also compares the differences in indicator lipids and polyunsaturated fatty acids (PUFA) between zooplankton and sediment trap organic matter. Chapter 6 summarizes the results and describes the major conclusions of the dissertation research.

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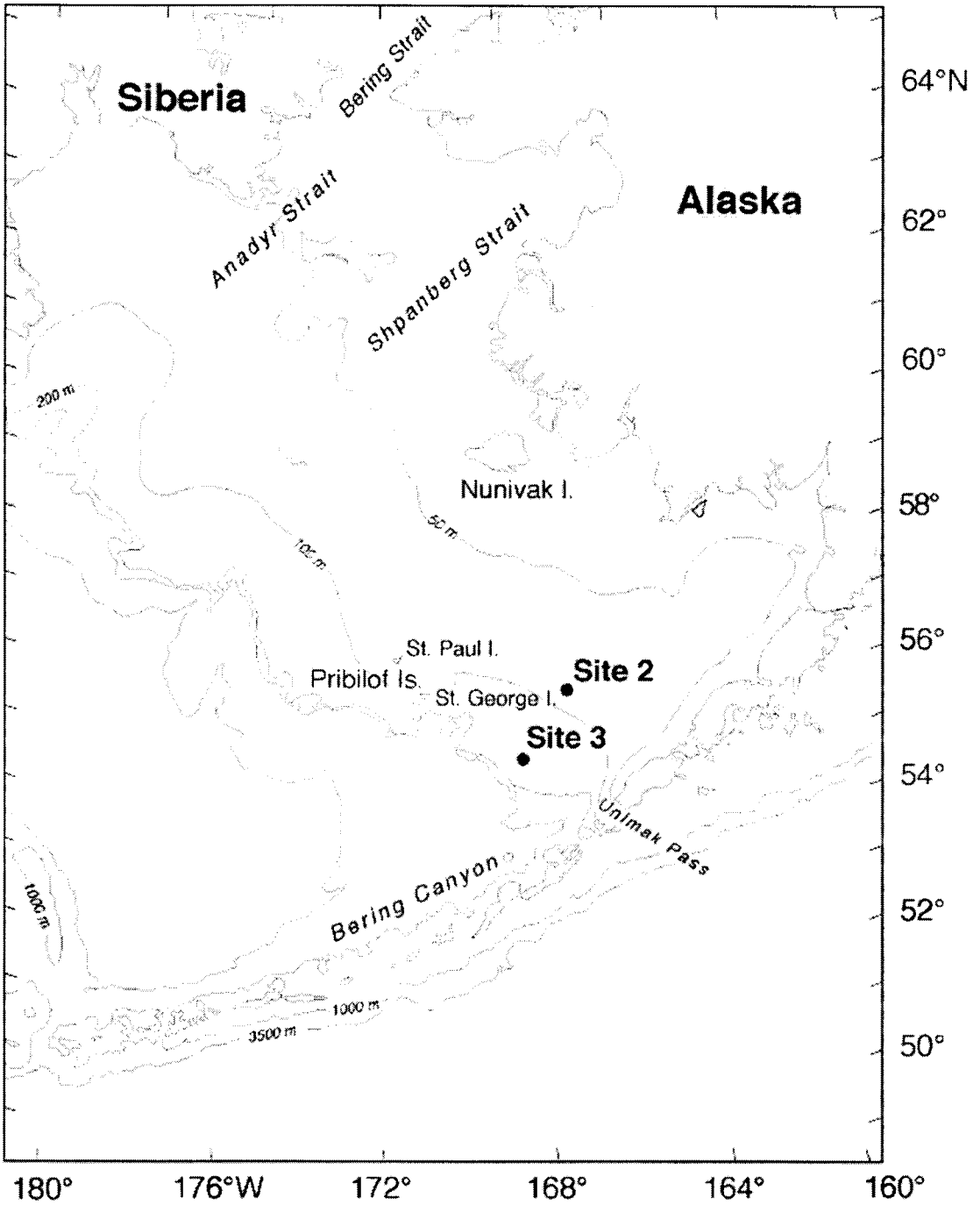

Figure 1.1. Location of the mooring sites on the southeastern Bering Sea shelf. Map courtesy of Phyllis Stabeno.

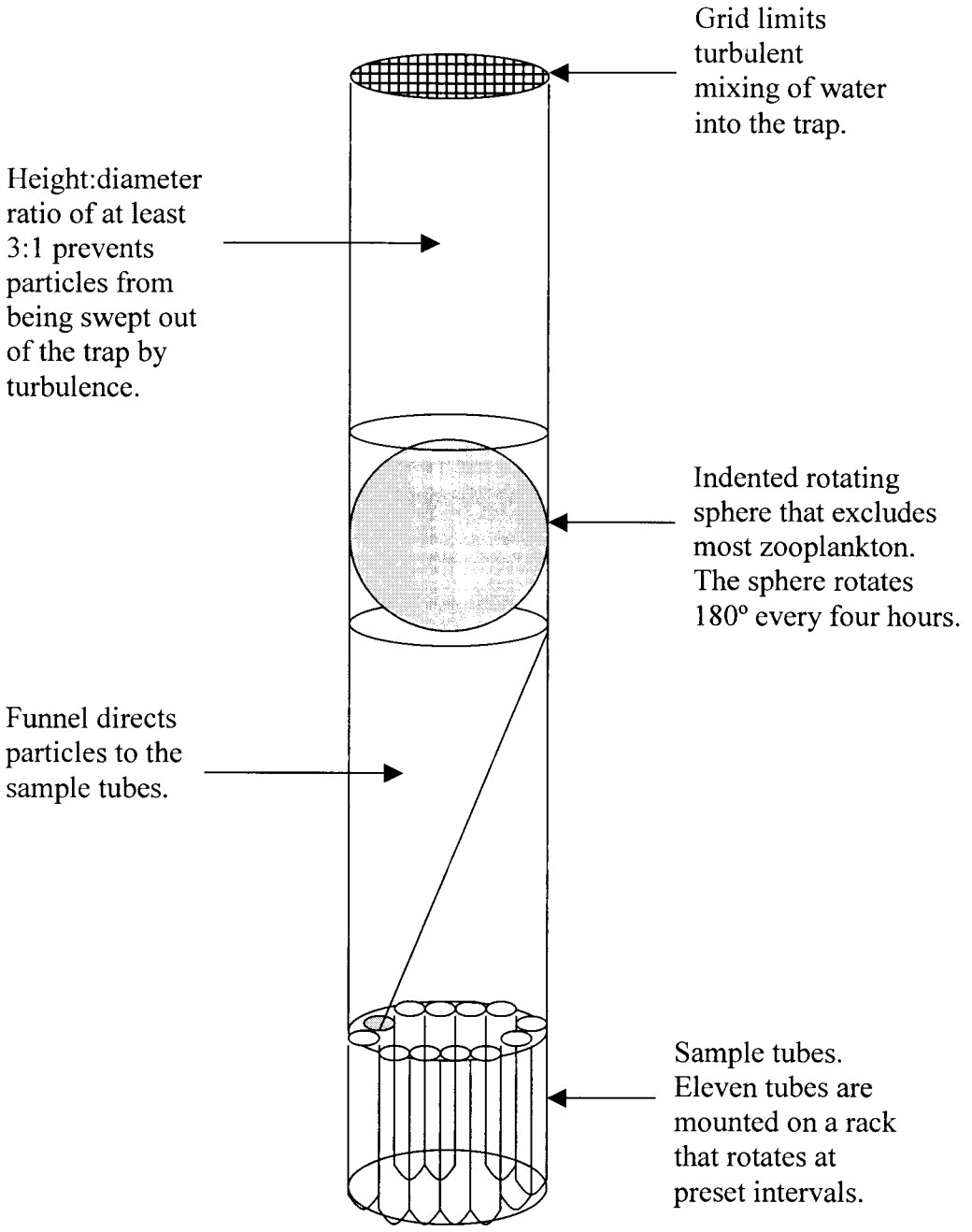

Figure 1.2. Schematic diagram of a sediment trap (after Peterson *et al.*, 1993).

Chapter 2.

Stable C and N isotopic composition of sinking particles and zooplankton over the southeastern Bering Sea shelf*

Keywords: SEBSCC, stable isotopes, particulate organic matter, sediment traps, zooplankton, USA, Alaska, Bering Sea

Abstract

Stable carbon and nitrogen isotopic composition of zooplankton, suspended particulate organic matter (SPOM), and sinking particles collected using sediment traps were measured for samples obtained from the southeastern Bering Sea middle and outer shelf during 1997 to 1999. The quantity of material collected by the middle shelf sediment trap was greater in both spring and late summer and fall than in early and mid summer. The $\delta^{15}N$ of SPOM, sinking material and zooplankton showed greater inter-annual variability at the middle shelf site (M2) than at the outer shelf site (M3). Zooplankton and sinking organic matter collected by M2 sediment traps became more depleted in ^{15}N from 1997 through 1999, associated with a change from unusually warm to unusually cold conditions. Suspended and sinking organic matter and zooplankton collected from M3 decreased only slightly in $\delta^{15}N$ from 1998 to 1999. SPOM, zooplankton, and sediment trap samples collected at M2 were usually enriched in $\delta^{15}N$ and $\delta^{13}C$ over those from M3. However, in 1999 sediment trap samples from the middle shelf were enriched in ^{13}C over M3 material, but the $\delta^{15}N$ of samples from the

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two sites was similar. The geographic pattern could be explained greater productivity over the middle shelf, associated with either isotopically heavy nitrogen being regenerated from sediments, or with utilization of a greater fraction of the available inorganic nitrogen pool during most years.

2.1. Introduction

The Bering Sea ecosystem supports some of the world's richest fisheries and large populations of marine mammals and seabirds, but shows marked interannual and interdecadal variability in productivity at high trophic levels (National Research Council, 1996; Hunt et al., 2002). Limited sampling in space and time has hampered characterization and especially understanding of the reasons for this variability. Most field research has been conducted in spring and early summer (e.g., Sambrotto et al., 1986; Whitledge et al., 1986; Niebauer et al., 1995), and there have been large temporal gaps in collection of many important types of data. To achieve significant improvements in understanding of the ecosystem, cost-efficient approaches that yield continuous information over long periods of time are needed. Moored sediment traps offer a means to examine temporal variability, on time scales of weeks to years, in the composition and quantity of sinking particles.

Stable nitrogen and carbon isotopic data have previously been used to investigate euphotic zone processes such as photosynthesis, nutrient uptake, particle regeneration via bacteria and zooplankton, particle removal from the euphotic zone by zooplankton repackaging, and higher trophic level dynamics. For example, Minagawa and Wada (1984) and Fry (1988) showed stepwise enrichment of $\delta^{15}N$ from primary producers to primary and secondary consumers. Altabet and Francois (1994) related nitrogen isotopic ratios of deep sea surface sediments to nitrate utilization in overlying surface waters. Wu et al. (1997, 1998) found a seasonal signal in the $\delta^{15}N$ and $\delta^{13}C$ values of sinking particulate matter in the northeastern Pacific Ocean, related to nutrient

uptake and regeneration. Rau (1989) found a strong inverse correlation between $\delta^{13}C$ enrichment of plankton and $[CO_2]_{aq}$ in the south Atlantic and Weddell Sea. On the other hand, Villinski et al. (2000) observed seasonal patterns of $\delta^{13}C$ in SPOM from the Ross Sea that were mainly related to aspects of phytoplankton dynamics, such as growth rate or species-specific fractionation. Laboratory research has demonstrated that $\delta^{13}C$ of phytoplankton is largely controlled by growth rates and cell size, rather than being a direct, simple function of $[CO_2]_{aq}$ (Laws et al., 1995; Popp et al., 1998).

This study aimed to elucidate inter-annual variations in the timing of primary production, nutrient availability, and zooplankton grazing. A time series of stable isotopic data from suspended particulate organic matter (SPOM), sinking material collected by sediment traps, and zooplankton provided indicators of these processes on a seasonal and inter-annual basis. This research is a component of SEBSCC (Southeast Bering Sea Carrying Capacity), a research program whose goals include increased understanding of the Southeast Bering Sea ecosystem and its carrying capacity for walleye pollock. The sediment traps used in this research were deployed at two sites on the Bering Sea shelf in conjunction with biophysical moorings, which collected current, fluorescence, temperature and salinity data (Stabeno et al., 2001; 2002). Collaborators measured carbon and nitrogen uptake rates and nutrient concentrations (Rho, 2000).

2.2. Materials and Methods

Two indented rotating sphere sediment traps, equipped with an eleven-sample carousel, collected a time series of sinking particles (Peterson et al., 1993). A unique

feature of this trap design is the indented rotating sphere that excludes swimmers from sample tubes. The traps were deployed at two sites on the Bering Sea shelf. The first site, referred to as M2, is located on the middle shelf (56°53'N, 164°02'W), where the water depth is 73 meters. The trap was deployed at 35 meters depth. The second trap, at site M3 (56°04'N, 166°20'W), was located over the outer shelf where the depth is 123 meters. The trap was deployed at 70 meters depth. The M2 trap has been deployed year-round since February 1997. The M3 trap was deployed from February through September in 1997 and 1998 and year-round in 1999. The traps were turned around twice a year, in February and September.

Prior to trap deployment, 5 g NaCl and 50 mg $HgCl_2$ were placed in the sample collection tubes (Lee et al., 1992). The duration of each sample collection interval was one to three weeks, depending upon projected flux to the traps. On retrieval samples were immediately stored in pre-combusted glass jars that had been baked at 460° C for 8 hours. Each jar lid was lined with acid-cleaned Teflon®. The samples were frozen until split for isotopic, microscopic and lipid analysis.

Sediment trap samples used for isotopic analysis were filtered using baked Whatman GF/F filters, then oven dried at 60° C and acid fumed. Once the samples were dry, the filters were cut in halves or quarters. Stable isotope analysis of sediment trap samples was performed using one of two instruments, the Europa Scientific Roboprep C/N Biological Sample Converter/20-20 Stable Isotope Analyzer, or the Carlo Erbo Autoanalyser Con Flo II Model NC 2500 with the Finnegan Mat Delta Plus Mass Spectrometer. Instrument precision was ± 0.26 ‰ for nitrogen and ± 0.1 ‰ for

carbon. A working standard (peptone) was run every 10 samples. The isotope ratios for nitrogen and carbon, relative to the standards air and PDB (PeeDee Belemnite), are reported as follows,

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where $X = {}^{15}N$ or ${}^{13}C$, and $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$.

Microscopic analysis was performed on each sediment trap sample using the inverted microscope method. A sub-sample of each sediment trap sample was dispersed into a combined plate-settling chamber; this consisted of a tall cylinder (approximately a 60 ml capacity) and a bottom-plate chamber (1.5 ml capacity). The chamber was filled with 25 ml of water and each sub-sample was allowed to settle into the bottom-plate chamber for 24 hours. After sedimentation, the cylinder was removed. The bottom plate chamber was fitted with a cover slip and placed into the mechanical stage of the Zeiss Telaval 31 inverted microscope. Phytoplankton cells were counted and identified until a total of 300 was reached; other particle types seen in each field viewed, such as fecal pellets, were also enumerated.

Plankton were collected by oblique tow in February 1997-1998, April 1997-1999, May 1999 and September 1999 aboard the NOAA Ship Miller Freeman R-223 using a 53 μm CalVET, and 153 μm and 333 μm bongo nets. Plankton samples were also collected aboard the R/V Wecoma in June 1997 and May 1998 and aboard the R/V Thomas G. Thompson in February 1999, using 153 μm and 333 μm bongo nets. Plankton were collected at each mooring site and at four surrounding stations. Plankton were also collected at a second, northern, middle shelf site, M4, when it was

ice-free. Upon collection, copepods, euphausiids, Scyphozoan medusae, and chaetognaths were sorted to genus or species by picking individual organisms under a dissecting microscope, placed in glass vials, and frozen immediately at -20° C. Plankton samples used for isotopic analysis were oven dried at 60° C for 24 hours and acid fumed. Between 1 mg and 1.5 mg of each sample was weighed using a Cahn 26 Automatic Electrobalance and submitted for isotopic analysis as described for the sediment trap samples. Reported means are the average of all zooplankton samples analyzed for the specified location and year. A t-test ($p=0.05$) was used to test the means for significant differences.

Bering Sea sediments were collected using a modified Soutar box corer during the May 1998 cruise aboard the *R/V* Wecoma. The top two centimeters of each core was frozen and stored for isotopic analysis. Sediments were acid treated with concentrated HCl, dried at 60° C for 24 hours, and then homogenized and weighed for isotopic analysis.

Water samples for SPOM analysis were collected in 5 or 10 liter Niskin bottles. The entire bottle was emptied into a polycarbonate carboy through 333 μm net to remove larger zooplankton. An aliquot of about 1 liter was filtered through a 25 mm Whatman GF/F precombusted glass fiber filter, and the filters were stored frozen until analyzed. After thawing and drying at 60° C, the filters were fumed with HCl vapors in a vacuum desiccator, dried, cut in sections, and analyzed as described earlier for stable isotopic analyses of sediment trap samples.

Nutrients were analyzed using standard methods modified for small volumes (Whitledge et al., 1981; 1986) using an ALPKEM RFA model 300 automated nutrient analyzer. February samples were stored frozen and returned to Fairbanks for analysis. For other sampling times analyses were completed on board ship.

2.3. Results

2.3.1. Zooplankton

Zooplankton collected over the middle shelf (M2, M4) were consistently enriched in ^{15}N relative to those collected near the outer shelf station M3, for all three years and for all taxonomic groups examined (Table 2.1). For example, in 1997, middle shelf copepods (*Calanus marshallae*) were more than 4‰ heavier than outer shelf copepods (*Neocalanus cristatus* and *N. plumchrus*), This difference continued in 1998 and 1999, but was smaller. Middle shelf euphausiids (*Thysanoessa raschi*) were almost 3‰ heavier than outer shelf euphausiids (*T. inermis*) in 1997, and, as for the copepods, the difference was less in 1998 and 1999. Chaetognaths (*Sagitta* spp.) and Scyphozoan medusae were also enriched in ^{15}N at M2 relative to M3. In addition, zooplankton collected at M2 and M4 were usually enriched in ^{13}C relative to those collected near M3 (Table 2.1). *Calanus* (M2) was enriched in ^{13}C over *Neocalanus* spp. (M3) throughout the study. *T. raschi* (M2) was enriched over *T. inermis* (M3), except in 1997, when their $\delta^{13}C$ values were equal.

Because there were only three to four sampling opportunities each year, and

because sometimes one of the species normally collected was absent from the sample, it was not possible to examine seasonal changes in detail. However, at M2 copepod $\delta^{15}N$ and $\delta^{13}C$ usually decreased by 1 to 2‰ between February and April, May, or June, and $\delta^{15}N$ usually decreased for other zooplankton also. Such trends were absent, except in copepod $\delta^{13}C$, at M3. For copepods and euphausiids at M2, annual means (Table 2.1, Mean 2) were recalculated by averaging the mean values for each sampling date, to eliminate any effect of different sample sizes at different sampling times during the year. However, means calculated in this way were nearly identical to those calculated by simple averaging of all data from the year (Table 2.1, Mean 1).

Zooplankton collected at M2 showed interannual differences in $\delta^{15}N$, especially between 1997 and 1998. The $\delta^{15}N$ values for *Calanus* and *T. raschi* were significantly greater in 1997 than 1998 or 1999 (Table 2.1). However, the $\delta^{15}N$ values of chaetognaths and Scyphozoa did not differ significantly between any two of the three years. Both middle shelf *Calanus* and *T. raschi* $\delta^{13}C$ increased significantly in 1999 relative to 1998. At M3 over the outer shelf, $\delta^{15}N$ values for euphausiids, chaetognaths and Scyphozoa were the same all three years. Only the copepod $\delta^{15}N$ varied significantly, decreasing by 1.5‰ from 1997 to 1998. Euphausiid $\delta^{13}C$ decreased significantly from 1997 to 1998. Outer shelf $\delta^{13}C$ values for copepods, chaetognaths, and Scyphozoa remained the same from 1997 through 1999.

2.3.2. SPOM and net plankton

As seen for the zooplankton, net plankton and SPOM collected in May 1998

were enriched in both ^{15}N and ^{13}C at M2 compared with M3 (Table 2.2). The limited number of samples from 1999 showed no difference in $\delta^{15}N$ at the two sites, but $\delta^{13}C$ was markedly lighter at M3. As seen for the copepod and euphausiid annual means at M2, the May 1999 $\delta^{13}C$ was heavier than that for May 1998. SPOM and net plankton $\delta^{15}N$ were markedly lighter in May 1999 than in May 1998 at both M2 and M3, but that difference was not seen in the zooplankton annual means (Table 2.1). The $\delta^{15}N$ of the SPOM, net plankton, and contemporaneous sediment trap samples were generally similar within variability at both M2 and M3. Except in May, 1999, sediment trap samples had consistently greater $\delta^{13}C$ than did SPOM and net plankton, at both sites.

2.3.3. Sediment trap samples

The amount of organic carbon collected by the sediment traps at M2 is shown in Figure 2.1, along with the cube of daily average wind speed at St. Paul Island (57°08'N, 170°18'W). St. Paul Island is about 350 km WNW of M2, but this is the only year-round wind record for the region. Since pressure areas have dimensions of about 1000 km in this region (Bond and Adams, 2002), Pribilof winds are likely a reasonable reflection of winds at the mooring site. The amount of material collected at M2 in 1998 was much greater than that in 1997 or 1999. All three years showed a similar annual pattern, with greater quantities in early spring and fall, and lesser amounts in summer and midwinter. The M2 data show some relationship with the wind patterns, i.e., the summer period of minimal collections also corresponds to the annual minimum in wind. This is not true of the winter minimum in collected material, however. Also, some of

the larger quantities collected were associated with relatively calm winds, for example, late April and July, 1997, early September, 1999, and late May and September-October, 1999.

The C/N ratio ranged from 4.8 to 9.0. There was a maximum in C/N in late May-early June during all three years (Figure 2.3). Local C/N ratio minima were associated with the April, 1997, July, 1997, September, 1997, March, 1998, and March-April, 1999, maxima in the amount of organic matter collected. The spring, 1998 C/N ratio of trapped material was less than that in 1997 or 1999.

The amount of material collected at M3 was much less in 1998 than in 1999 (Figure 2.2) and less in both years than the quantity collected at M2. In 1998, the amount collected was less than 10 mg C/m^2 day except in March and April, when 50 to 100 mg C/m^2 day were trapped. The maximum value in July, 1999, exceeded 300 mg C/m^2 day, and more than 50 mg C/m^2 day was collected at most times during 1999. The C/N ratio ranged from 4.5 to 7.3, and high values were found in late May-early June as at M2 (Figure 2.3). However, the C/N ratio was also high in April of 1999.

The means of $\delta^{15}N$ and $\delta^{13}C$, weighted by the quantity of organic matter collected by the sediment traps, are given in Table 2.3. The mean for April and May sample collections is similar in all cases to the mean for April through August. The average $\delta^{15}N$ was 3‰ greater at M2 than M3 in 1997 and 1998, but the two sites had similar mean values in 1999. The $\delta^{13}C$ was 2 to 3‰ lighter at M3 than at M2 during both 1998 and 1999. The mean $\delta^{15}N$ at M2 was about 3‰ greater in 1997 and 1998

than in 1999. The mean $\delta^{13}C$ was similar for all three years at M2, but was about 0.5‰ heavier in 1998. M3 $\delta^{15}N$ averaged about 1‰ heavier in 1998 than 1999. The spring mean $\delta^{13}C$ was identical in 1998 and 1999, but the average for the spring-summer period was 0.6‰ lower in 1998 than 1999.

The $\delta^{15}N$ of sinking organic matter at M2 showed similar seasonal pattern of variation in all three years, an increase in late winter and spring, reaching a maximum in late April (1998 and 1999) to early June (1997), followed by a decrease into summer (Figure 2.4). In 1997 and 1998 the decrease continued through fall, but in 1999 the values increased again after June and were greater than 13‰ in October. In 1998, a high percentage of fecal material in February-April samples was associated with high $\delta^{15}N$ values. The early 1998 sediment trap samples also contained a notably large amount of amorphous organic material. The $\delta^{15}N$ of spring and summer 1999 M2 sediment trap material was about 4‰ lower than that collected during 1997 and 1998, as also seen for the May 1999 SPOM and net plankton samples.

The M2 $\delta^{13}C$ exhibited a seasonal pattern similar to that of $\delta^{15}N$, with higher values in late winter, and a decrease through spring and summer (Figure 2.5). In 1997 and 1998 the decrease continued into fall and early winter, but in 1999, as was the case for $\delta^{15}N$, the $\delta^{13}C$ increased in August through November. By late winter, $\delta^{13}C$ had decreased to the average values of previous years.

No sediment trap samples were collected at M3 in 1997. Both the $\delta^{15}N$ and $\delta^{13}C$ of sinking material at M3 showed a pattern of higher values in late winter and

decreasing values into the fall of 1998, similar to the pattern at M2. This pattern did not hold in 1999, however, when the $\delta^{15}N$ and $\delta^{13}C$ had no seasonal trend. In 1998, samples from February 26 and May 21 recorded unusually low $\delta^{15}N$ and $\delta^{13}C$ values. The amount of carbon collected in these samples was very low and the C/N ratios were 7, greater than those of most other samples. As was true for zooplankton and SPOM, sediment trap samples collected at M3 were depleted in ^{15}N and ^{13}C compared with those collected at M2 in 1998 and most of 1999. In 1999 the $\delta^{15}N$ values at the two sites were equal in July, August, and December (Figure 2.4).

2.3.4. Sediment cores

The $\delta^{15}N$ and $\delta^{13}C$ of sediments (0-2 cm) collected from middle shelf stations near M2 averaged 7.6‰ and -21.85‰, respectively, at M2 and 6.7‰ and -23.1‰ at M4. The isotopic composition of M3 sediment was similar, with an average $\delta^{15}N$ of 6.7‰ and $\delta^{13}C$ of -21.00‰.

2.3.5. Nutrients

The February 1997 and 1998 nitrate concentrations at M2 were very similar, from 12 to 14 μM throughout the water column; they were slightly lower, 9-11 μM, in February 1999 (Figure 2.6). (February ammonium data are unavailable). In 1997 euphotic zone nitrate was depleted at M2 by early May, and in June there was substantial utilization of nitrate below the pycnocline, reducing the concentrations near the bottom to only 2 μM. The ammonium concentration, which was 8 to 10 μM in early May, decreased to less than 1 μM in surface waters by late June. The 1997 data

contrast sharply with May 1998 when the nitrate concentration was about 11 to 12 μM throughout the water column, only slightly less than February and April values. However, the ammonium concentration had increased markedly over April values, to 6 - 10 μM. On May 2, 1999, nitrate concentrations were 9 to 11 μM throughout the water column. This water sample was taken prior to the advance of ice over the mooring site about May 7, which prompted a bloom (Rho, 2000). The May 1999 ammonium concentration was much less than that in 1997 and 1998.

No February data are available for M3. The early May 1997 nitrate concentrations were 3 to 6 μM in the mixed layer, but by mid-June, surface water nitrate had decreased to 0 μM (Figure 2.7). Ammonium increased throughout the water column between May and mid-June, but by late June, it was almost depleted near the surface. The1998 nutrient profiles at M3 were similar to those at M2; nitrate was high, 14-15 μM, as late as mid-May. In early May 1999, the nitrate concentration was about 18 μM to 70 m depth, but at 120 m depth the nitrate concentration was 28 μM, indicating an influx of nutrients from slope water.

2.4. Discussion

2.4.1. Sediment traps and the quantity of material collected

In the upper ocean, swimmers (zooplankton which enter traps actively rather than by passive sinking) often constitute most of the collected material, and are very difficult to quantitatively separate from other particles (Lee et al. 1988; 1992).

However, swimmer-excluding traps (Peterson et al., 1993) were used in this study. Samples were carefully examined for intact zooplankton, but these were usually absent. Occasionally, one or two small copepods were found and picked out. In a single event, a large number of pteropods was present in the M2 September 1999 sample. Pteropods were extremely abundant in the water at that time, and it's uncertain whether the trapped animals were swimmers or sank into the trap.

Sediment traps often do not collect sinking particles quantitatively, and the shallow southeast Bering Sea sites where the moorings were deployed are not ideal for quantitative particle trapping. Even in favorable locations (deep water with relatively weak currents), radioisotopic calibrations indicate that trapping efficiency is often not 100% and that under-trapping is most common (Buessler, 1991; Cochran et al., 1993). Tidal currents over the Bering Sea middle shelf can approach 20 cm sec^{-1}, although net currents are much slower, and this could affect efficiency (Gardner et al., 1983; 1996). Also, particularly for the middle shelf trap, resuspended bottom sediment is a potential contributor to the samples, though there is no evidence that it was a major component during most of the year. For example, the $\delta^{15}N$ of the underlying sediment at M2 (7‰-8‰), collected with a box corer, was much less than that of typical sediment trap samples. When resuspended sediment was expected, after very severe winter storms, M2 sediment trap samples contained numerous diatom fragments. This differed notably from the typical sample, in which intact diatom frustules, intact or broken fecal pellets, amorphous aggregates, and sometimes coccoliths were the major identifiable components. M3 samples never contained predominantly broken diatom tests. Given

the uncertainties in trapping efficiency and the potential for collection of resuspended sediments, at least after severe storms, we do not claim that the quantity of organic matter collected by the traps was equal to the vertical flux of sinking particles. Rather, this paper emphasizes the composition of the sediment trap samples and how temporal and spatial variations in collected material relate to conditions in the water column.

Some especially severe storm events did correspond to unusually high amounts of material collected by the M2 trap (Figure 2.1). Sustained wind speeds in excess of 15 m/sec (3375 m^3/sec^3) were associated with large collections of organic material during February through April, 1998 and 1999 and November 1998. Substantial primary production is unlikely at these times except, perhaps, in April. In November, 1999, both microscopic and lipid analyses indicated that the material collected consisted mainly of intact diatoms, which suggests that if the collected material was resuspended, it consisted of recently settled phytoplankton. In February through April of 1998 and 1999, the collections had numerous intact fecal pellets in addition to amorphous material, again suggesting resuspension of a recently deposited layer.

However, high rates of organic matter collection by the M2 trap also occurred at times when winds were relatively calm. These include late April of 1997, late April-early May of 1998, and late May of 1999, probably associated with spring phytoplankton blooms. In July of 1997, a small wind event during an otherwise very calm period was associated with increased particle collection by the M2 trap, likely due to productivity spurred by nutrients supplied to surface waters via wind mixing (Sambrotto et al., 1986). There were also late summer-early fall maxima in the quantity

collected, associated with moderate winds, which were probably due to increased fall productivity associated with mixing and nutrient influx to the photic zone. Some of these fall samples had very high numbers of coccoliths, but diatoms and fecal pellets were also numerous. The data suggest that fall blooms are important contributors to annual primary production in the southeastern Bering Sea.

One approach to assessing the quantity of the material the sediment trap collected is to compare it to rates of carbon, nitrate, and ammonium uptake by phytoplankton. As yet, data are available only for 1997 and 1998 (Rho, 2000). The measurements were made almost entirely in the spring, and primarily at times and places where phytoplankton biomass (chorophyll) was low. Measured carbon and ammonium uptake rates over the middle shelf were much greater in 1998 than 1997, as was the amount of material collected by the sediment trap. However, there was no clear difference in nitrate uptake rate, either as measured by a tracer or as estimated by disappearance of nitrate from the mixed layer during the spring and summer.

Under the conventional paradigm for the open ocean (Eppley et al., 1979), the amount of nitrogen collected by the sediment trap should equate to the amount of nitrate removed from the photic zone. For late April through August, 1997, the sediment trap collection was about 2.2 g N m^{-2}, while the estimated nitrate consumption for April through August was 58 g N m^{-2} (Rho, 2000), greater as expected because the trap was not deployed for the whole period nor at the time (the first part of April) when most nitrate consumption occurred. Further, because of the unusually deep nutricline in summer, 1997, some of the nitrate uptake and primary production occurred below the

depth of the trap. In 1998, 33 g N m^{-2} were collected during April through August, less than but comparable to the 53 grams of nitrate nitrogen uptake (Rho, 2000). However, there was also substantial ammonium consumption during this period, at rates more than ten times the nitrate uptake rate. Because a substantial quantity of ammonium appeared between April and May without an equivalent net consumption of nitrate, it appears to constitute a new, rather than regenerated, nitrogen input to the pelagic system. The amount of material collected from April through August, 1999, was similar to that in 1997, 3.4 g N m^{-2}. It is not entirely clear why the amount of material collected by the M2 trap in 1998 was so much greater than that collected in 1997 or 1999. Monthly mean wind speed[3] was unusually high in April and August of 1998 relative to the other two years, but the mean values were similar and low for May through July of all three years. High winds in spring, 1998, were associated with high concentrations of nitrate in surface waters that persisted through May (Figure 2.6). The other unusual conditions in spring, 1998, were the high ammonium concentrations (Figure 2.6) and consumption rates (Rho, 2000). Hence, there is some evidence that the nutrient supply to the photic zone was greater in 1998 than in the other two years.

2.4.2. Cross-shelf variation of the stable isotopic composition of SPOM, plankton, and sediment trap samples

The greater middle shelf $\delta^{15}N$ of SPOM, zooplankton, and particles collected by sediment traps, relative to that found for outer shelf samples, reflects variations in the isotopic composition of phytoplankton that ramify throughout the food web. Schell et al. (1998) reported a similar pattern in zooplankton $\delta^{15}N$ across the Bering Sea shelf,

which they attributed to progressive cross-shelf nutrient depletion. Because phytoplankton preferentially assimilate the lighter isotope, the residual inorganic nitrogen pool becomes progressively heavier as it is consumed (Wada and Hattori, 1981; Altabet, 2001). Previous work has shown that the main source of nutrients for the southeastern Bering Sea shelf is the high-nutrient water overlying the bordering continental slope. Calculated tidally-driven horizontal diffusive nutrient fluxes appear to be large enough to provide the nitrogen required to sustain shelf primary production (Coachman and Walsh, 1981; Whitledge et al., 1986). Horizontal advection of nutrients was estimated to be small (Coachman, 1986), but recent observations of rapid but intermittent cross-shelf flows indicate the potential for advective transport as well (Stabeno et al., 2002). As a consequence of the offshore nutrient source, the middle shelf can be described as nutrient depleted, while outer shelf nutrients are more rapidly replenished by the on-shelf transport from slope water (Hattori and Goering, 1981; Whitledge et al., 1986).

However, both the outer shelf site and the middle shelf site M2 typically exhibit complete depletion of surface water nitrate during the spring bloom (Whitledge et al., 1986; Rho, 2000). If the initial nitrate $\delta^{15}N$ at the two sites was the same, the weighted average $\delta^{15}N$ of sediment trap particles over the productive season (Table 2.3) should be the same also, but that was observed only in 1999. One potential reason for the difference between M2 and M3 in 1997 and 1998 was that, while surface water nitrate is ultimately depleted at both sites, nitrogen utilization, estimated based on the quantity of organic material collected by the sediment traps (Figures 2.1 and 2.2), was greater at

M2. The additional nitrogen apparently came from below the thermocline at M2, ultimately substantially decreasing nitrate and ammonium concentrations throughout the water column. At the outer shelf site (M3) subsurface waters can mix more readily with nutrient rich waters offshore, adding fresh (and isotopically lighter) nitrate throughout the productive season. Hence, nitrate supplied to surface waters by vertical mixing would not become enriched in ^{15}N, as probably occurs at M2. Another possible explanation is that the initial isotopic composition of the inorganic nitrogen supplied at the two sites differed. Since the isotopic composition of nitrate supplied from offshore should have been uniform, and coastal waters are not a likely nitrogen source, isotopically heavy inorganic nitrogen would have to be supplied on the shelf, perhaps via regeneration from sediments. Regenerated ammonium should be isotopically lighter than the decomposing or digested organic matter (Checkley and Miller, 1981), but if material decomposing at the sediment-water interface in the winter and spring were similar to the isotopically heavy sediment trap samples collected during 1997 and 1998, the released inorganic nitrogen could have a $\delta^{15}N$ of about 10‰.

The $\delta^{15}N$ also indicated differences in trophic level of certain organisms between the middle and outer shelf regions. At M2, chaetognaths were enriched 2-5‰ over both copepods and euphausiids for the period 1997-1999. At M3, chaetognaths were heavier by 3-4‰ than euphausiids, which were enriched 1-1.5‰ over copepods. This is consistent with a more omnivorous diet for euphausiids at M3.

Geographic trends for $\delta^{13}C$ were similar to those for $\delta^{15}N$. Middle shelf zooplankton were usually enriched in ^{13}C over their outer shelf counterparts, and the

particulate matter collected by sediment traps had consistently greater $\delta^{13}C$ at M2 than at M3. This cross-shelf $\delta^{13}C$ trend is opposite to that reported by Schell et. al (1998), who attributed a pattern of progressively lighter $\delta^{13}C$ of copepods from the outer to the inner shelf to decreasing primary productivity associated with nutrient depletion. As discussed in greater detail later, $\delta^{13}C$ of phytoplankton is affected by many factors, of which growth rate is only one. However, the data reported here are consistent with substantially greater rates of primary production over the middle shelf than the outer shelf. In particular, the sediment trap at M2 collected substantially more organic matter than the trap at M3, and SPOM, zooplankton, and sediment trap particles all had greater $\delta^{13}C$ at M2 also.

2.4.3. Seasonal and inter-annual variation of the stable isotopic composition of SPOM, plankton, and sediment trap samples

In 1997, the Bering Sea had much less cloud cover and higher atmospheric and water temperatures than normal. In early spring, sea ice was present over M2 and the retreat of sea ice early April resulted in melt-water salinity stratification of the water column and an ice-edge bloom (Stabeno et al., 2001). The bloom ended when nutrients were stripped from the surface layer by late April (Figure 2.6), and from late April through late May little regenerated production occurred, since ammonium was depleted as well (Rho, 2000). Therefore, net plankton collected in June, sinking organic matter, and zooplankton were all enriched in ^{15}N (Tables 2.1 and 2.3; Figure 2.4). Microscopic analysis showed that the April sediment trap sample consisted mainly of sinking

diatoms. At the end of May, the C/N ratio of sinking material was 9, and $\delta^{15}N$ values had increased to greater than 15‰.

By June, a subsurface phytoplankton bloom depleted nitrate at depth (Stockwell et al., 2001). Slightly lower June $\delta^{15}N$ values in sediment trap material may correspond to phytoplankton growth near the base of the euphotic zone. In September fall mixing commenced, which brought nutrients to the surface and coincided with the sinking of coccoliths into the sediment trap. Owing to new nitrate input, the $\delta^{15}N$ decreased in the September samples (Figure 2.4), which contained diatoms and fecal material in addition to the coccoliths. A coccolithophorid bloom had been present over the middle shelf since July, and SeaWIFS imagery showed that the bloom covered most of the middle and outer shelf south of Nunivak Island in September (Stockwell et al., 2001).

The $\delta^{15}N$ of SPOM and sinking organic material collected in 1998 was initially high, similar to that in 1997. Nutrient concentrations and their temporal variation differed markedly from 1997, with nitrate concentrations in surface waters remaining high through May. Much more organic matter was collected by the sediment traps in 1998 than in 1997, and microscopic analysis of the trapped material revealed numerous fecal pellets. Zooplankton feces are isotopically enriched compared with their food, but can be either heavier or lighter than their bodies (Checkley and Entzeroth, 1985; Checkley and Miller, 1989; Altabet and Small, 1990). However, in 1998 the sediment trap particles were a remarkable 3-4‰ heavier than the copepods and euphausiids, rather than being similar as they were in 1997. Detrital aggregates and unidentified flagellates, most likely heterotrophs, were also found in abundance. Many

heterotrophic flagellates are known to be bacteriovores that attach to sinking detrital matter (Caron, 1991). Since the water column's heat content was higher than ever previously recorded (Stabeno et al., 2001) rates of bacterial decomposition may have been accelerated (Rho, 2000), resulting in elevated flagellate biomass. Consistent with this idea, the ammonium concentration across the middle shelf was elevated in early spring (Rho, 2000). The abundance of fecal matter in the sediment trap samples suggests that zooplankton grazing was also an important factor in high ammonium concentrations. As in 1997, the $\delta^{15}N$ of sediment trap samples decreased in fall, 1998, associated with increased vertical mixing and new nutrient supply to surface waters.

In 1999, the $\delta^{15}N$ of SPOM (Table 2.2) and sinking organic matter (Figure 2.4) was much lower than in 1997-98, although that of zooplankton was essentially unchanged from 1998 (Table 2.1). A lower flagellate to diatom ratio and less fecal material and detritus were observed in all 1999 spring and summer samples, compared with those from 1997 and 1998, consistent with the difference in $\delta^{15}N$. Another important difference was the relatively low ammonium concentrations in spring, 1999, compared with 1997 and 1998 (Figure 2.6). As discussed in the previous section, if the source of some of this ammonium were winter regeneration from sediment organic matter with high $\delta^{15}N$, it could explain the heavier $\delta^{15}N$ of sediment trap material in 1997 and 1998. The lower ammonium in 1999 could have resulted from unusually cold bottom water temperatures during spring (Stabeno et al., 2002). The $\delta^{13}C$ and $\delta^{15}N$ both decreased from February through the spring and summer, as in earlier years. But

in fall 1999, $\delta^{15}N$ values were higher than in February. An alternative explanation is that the $\delta^{15}N$ of nitrate supplied from the outer shelf was lower in 1999; the slightly lower nitrate $\delta^{15}N$ at M3 suggests that this could have been a factor, but it can't account for the much larger change at M2. Also counter to this idea is the fact that the bottom water salinity at M2 was nearly the same in spring of each of these years (Stabeno et al., 2002).

M3 was also nutrient depleted in 1997 (Figure 2.7), but zooplankton $\delta^{15}N$ values were much less than those at M2. In 1998, similar to M2, nutrient profiles show high nitrate concentrations in mid-May throughout the entire water column. The $\delta^{15}N$ of SPOM collected in early May was equal to that of sinking material, as was also observed at M2. Large fecal pellets from *N. cristatus* and *N. plumchrus* and flagellates composed a major portion of the sinking organic matter in March through May 20. The 1998 sediment trap samples were slightly more enriched in ^{15}N than in 1999 (Table 2.3). Much less material, especially diatoms, sank into the trap at M3 compared with M2, and in contrast to M2 the amount of material collected by the sediment trap at M3 was less in 1998 than 1999. More phytoplankton were present in the 1999 trapped material.

The $\delta^{13}C$ values of M2 copepods and euphausiids were slightly heavier in 1999 than in 1997 and 1998, while the weighted average of spring-summer sediment trap samples was slightly heavier in 1998 than in 1997 or 1999. The latter is consistent with the greater quantity of organic matter collected in 1998, if $\delta^{13}C$ reflects primary

productivity. However, if this were the case, consistency between the zooplankton and sediment trap samples would be expected. The $\delta^{13}C$ of M3 copepods and euphausiids was statistically the same in 1998 and 1999, while the weighted mean of sediment trap samples was slightly heavier in 1999. Again, the latter is consistent with the greater quantity of organic matter collected in 1999, if $\delta^{13}C$ reflects primary productivity, but a corroborating pattern in zooplankton is lacking. However, zooplankton sampled mainly in spring don't represent processes over the entire time frame sampled by the sediment traps.

Schell et al. (1998) reported zooplankton $\delta^{15}N$ and $\delta^{13}C$ for the Bering, Chukchi, and Beaufort Seas, from samples collected during 1985-1990 and 1993-1995. In his report, data from the outer shelf of the southeastern Bering and the middle shelf of the northern Bering Sea are combined as an "Eastern Bering Sea" region. The mean for n=64 copepod samples in this region was $9.8\pm0.22‰$, and for 33 euphausiids was $10.0\pm0.22‰$, lighter than the mean of our 1997 middle and outer shelf samples, but comparable to the 1998 and 1999 values. This is consistent with a variety of indicators that 1997 nutrient depletion was unusual compared to all other years sampled. Our 1997-1999 chaetognath data, however, are all distinctly heavier than those of Schell et al (1998), which averaged $12.9\pm0.30‰$ for their northern middle and southern outer shelf stations. This suggests that 1997-1999 chaetognaths were feeding at a higher trophic level than before.

Our M3 copepods are distinctly lighter in $\delta^{13}C$ than those reported by Schell et al. (1998), which averaged $-22.2\pm0.18‰$ for 72 samples from the eastern Bering. Our

$\delta^{13}C$ values for euphausiids and chaetognaths were similar to those reported by Schell et al. (1998), however. Schell (2000) reported a decreasing trend in the $\delta^{13}C$ of bowhead whale baleen over the past 30 years, which he attributed to decreasing primary productivity in the Bering Sea. The decreased copepod $\delta^{13}C$ in 1997-1999, compared with that of copepods collected during 1985-1990 and 1993-1995, is consistent with his observation, but the trend is not necessarily due to primary productivity changes. If the difference was due to a change in phytoplankton $\delta^{13}C$, it should have been seen in euphausiids and chaetognaths also.

The controls on $\delta^{13}C$ of phytoplankton have been investigated in culture (Laws et al., 1995; Popp et al., 1998). Phytoplankton carbon isotopic fractionation is a function of the ratio of algal growth rate to the concentration of dissolved carbon dioxide, $\mu/[CO_2]_{aq}$, for particular species. As growth rate μ increases or $[CO_2]_{aq}$ decreases, carbon isotopic fractionation decreases, leading to cells with increasing $\delta^{13}C$. However, the slope of this function varies more than twenty fold for different phytoplankton species, according to the ratio of cell surface area to volume. Although cell geometry as well as size is important, for cells with similar shapes, carbon isotope fractionation is greater for small cells. Hence their $\delta^{13}C$ is lower than that of large cells at a given $\mu/[CO_2]_{aq}$. In the field, μ , $[CO_2]_{aq}$, cell surface area/volume, and the $\delta^{13}C$ of the inorganic carbon pool can all vary temporally and spatially (Popp et al., 1998; Villinski et al., 2000).

Our cross shelf patterns in zooplankton, SPOM, and sediment trap samples, and seasonal changes in sediment trap samples, show very consistent positive correlations between $\delta^{13}C$ and $\delta^{15}N$, opposite to the trend reported by Schell et al. (1998), but similar to patterns observed by Goering et al. (1990) and Wu et al. (1999). As yet we have no definitive explanation for the spatial and temporal patterns of stable carbon and nitrogen isotope composition. Our data are consistent with any of the following interpretations.

First, higher $\delta^{15}N$ in spring relative to fall, over the middle shelf compared with the outer shelf, and during 1997 and 1998 compared with 1999, were mainly due to isotopically heavy ammonium regenerated from sediment organic matter during winter and spring. This would be an added nutrient source for the year being examined, although derived from earlier years, and hence could drive elevated rates of primary production and higher $\delta^{13}C$.

Second, high rates of primary production resulted in decreased $[CO_2]_{aq}$, causing the ratio $\mu/[CO_2]_{aq}$ to increase and isotopic fractionation to decrease, and leading to increased $\delta^{13}C$ of phytoplankton. The $\delta^{15}N$ of phytoplankton increased as fractional utilization of the available nitrate (or nitrate plus ammonium) pool increased, but, at least while the bulk of the organic matter production was occurring, phytoplankton growth rates and $\delta^{13}C$ remained high. For example, conditions that favored utilization of more of the total water column inventory of dissolved inorganic nitrogen at M2 could increase μ, $\delta^{13}C$, and $\delta^{15}N$. Such conditions include those in 1997, when utilization of

most of the nitrate below the thermocline occurred, and 1998, when increased mixing supplied more nutrients from subsurface to surface waters.

Third, temporal and spatial changes in phytoplankton species composition could also result in the observed patterns. In this scenario, smaller cells with greater surface area/volume ratio are more important primary producers over the outer shelf than over the middle shelf and in fall compared with spring. Such consistent patterns in phytoplankton species composition were not apparent from the microscopic examination of sediment trap samples, i.e., the species composition was highly variable in time and space. Therefore, one or both of the first two explanations is more likely.

2.5. Conclusions

- The quantity of material collected by the sediment traps was greater in both spring and late summer-fall than in early and mid summer. In spring, greater quantities of material collected were associated with increases in phytoplankton production and draw down of nutrients. The increased quantity of organic matter in sediment traps in late summer and early fall coincided with increasing wind mixing and supply of nutrients to the photic zone.
- The $\delta^{15}N$ and $\delta^{13}C$ values of SPOM, sediment trap material and zooplankton from the middle shelf site were usually heavier than sediment trap material and zooplankton from the outer shelf. This pattern could be explained greater productivity over the middle shelf, associated with either isotopically heavy

nitrogen being regenerated from sediments, or with utilization of a greater fraction of the available inorganic nitrogen pool.

- Seasonal and inter-annual variability of $\delta^{15}N$ at the middle shelf site M2 were pronounced. Interannual and seasonal changes in nutrient supply are probably responsible for much of the variability in $\delta^{15}N$.
- Our data are consistent with a relationship between $\delta^{13}C$ and primary productivity, and hence lend some support to the Schell (2000) inference that decreasing $\delta^{13}C$ of bowhead whale baleen indicates declining Bering Sea productivity over the past 30 years. However, we can't rule out other factors influencing $\delta^{13}C$, such as phytoplankton species effects.

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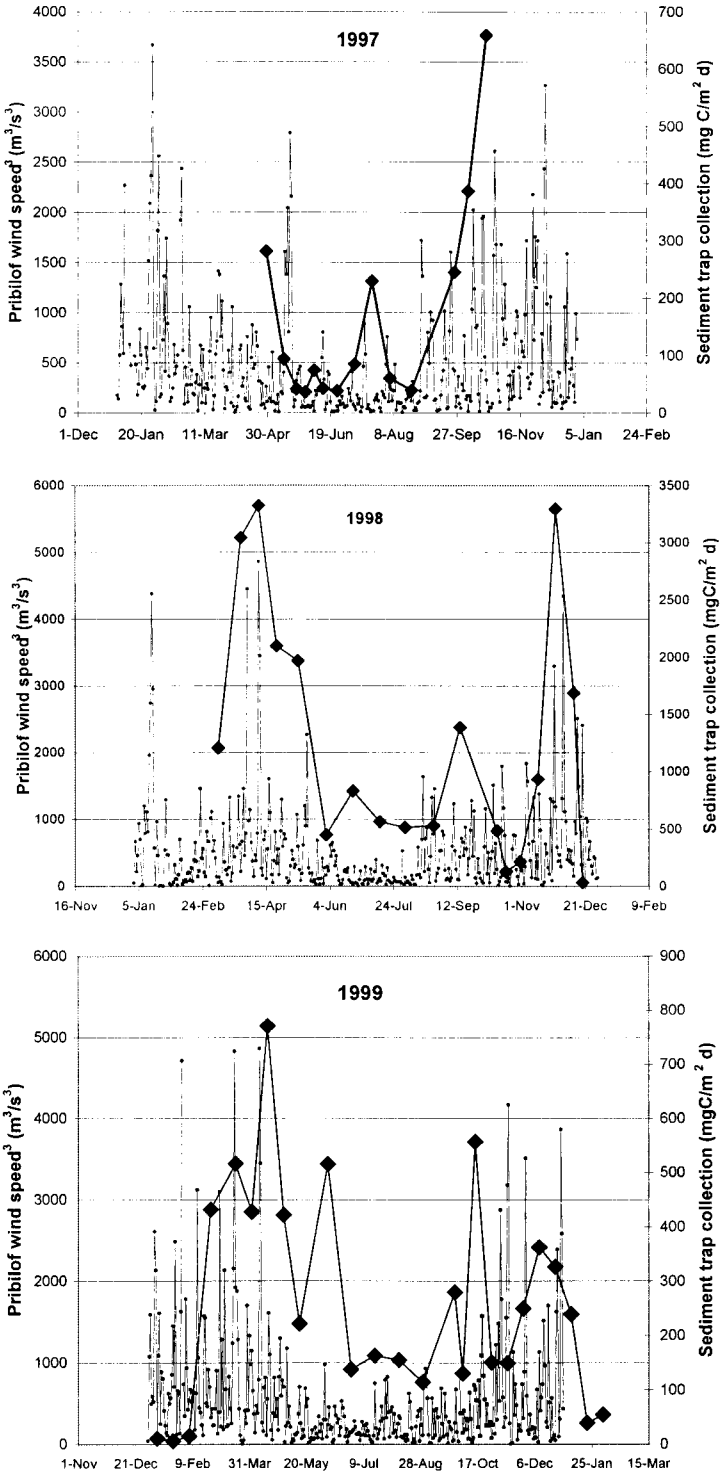

Figure 2.1. Pribilof wind speed^3 compared with the quantity of particulate organic carbon collected by sediment traps at the middle shelf site M2 for the years 1997 through 1999.

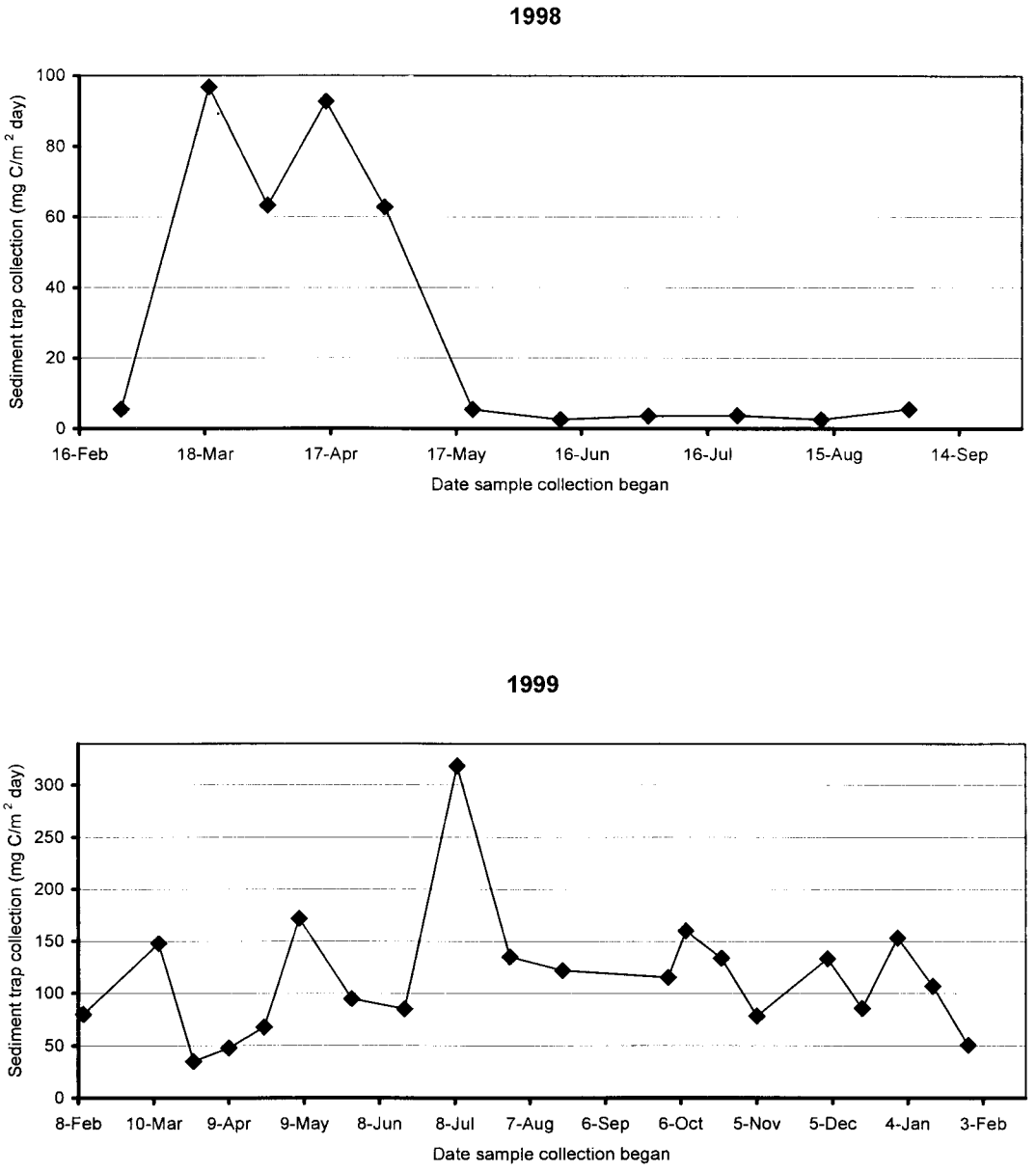

Figure 2.2. The quantity of organic matter collected by sediment traps at the outer shelf site M3 for the years 1998 and 1999.

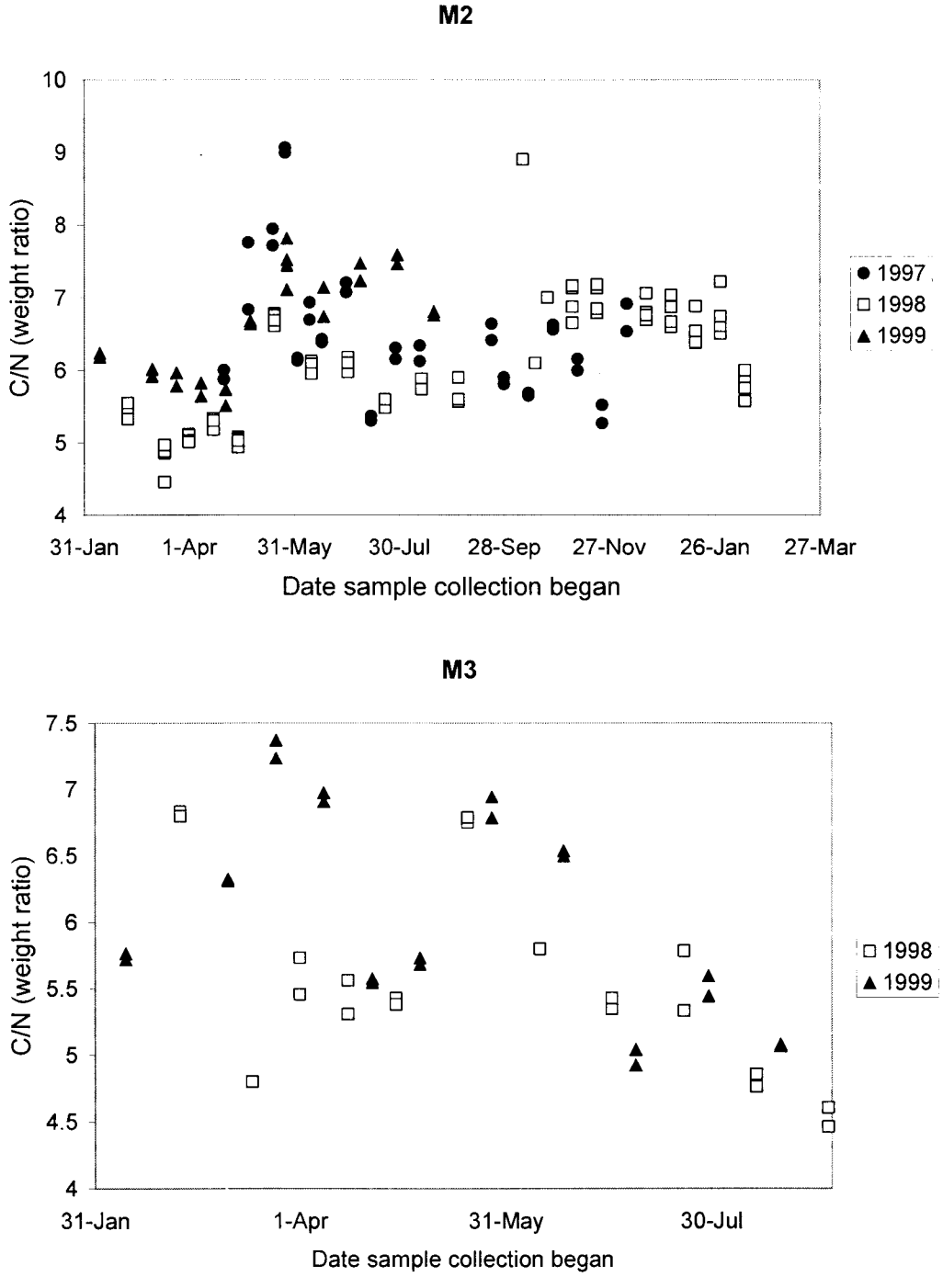

Figure 2.3. C/N (weight ratio) of organic matter collected by sediment traps located over the middle (M2) and outer (M3) shelf of the southeastern Bering Sea.

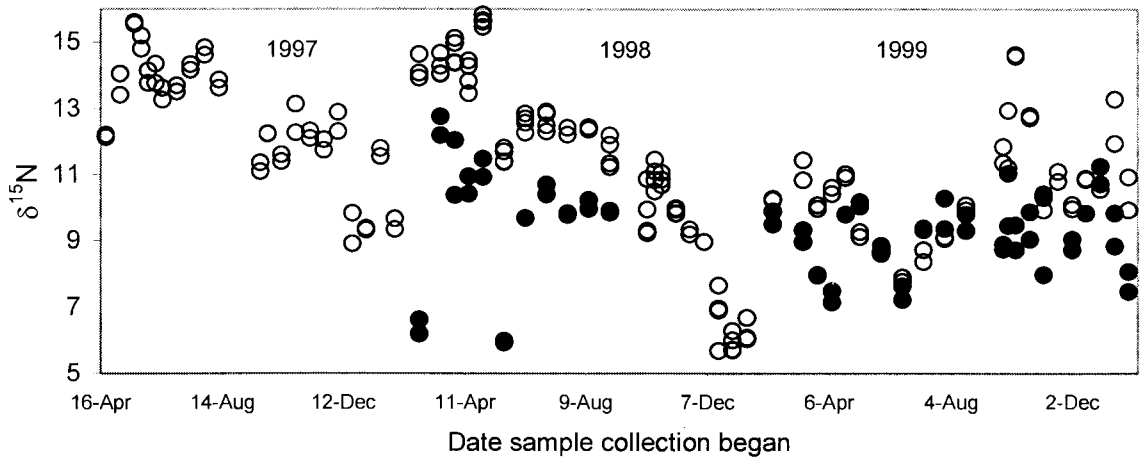

Figure 2.4. $\delta^{15}N$ (‰) of sediment trap samples collected at the middle shelf site M2 (o) and the outer shelf site M3 (•), April 1997 through January 2000. No samples were collected at M3 during 1997. Multiple symbols at a single time represent replicate analyses of a single sediment trap sample.

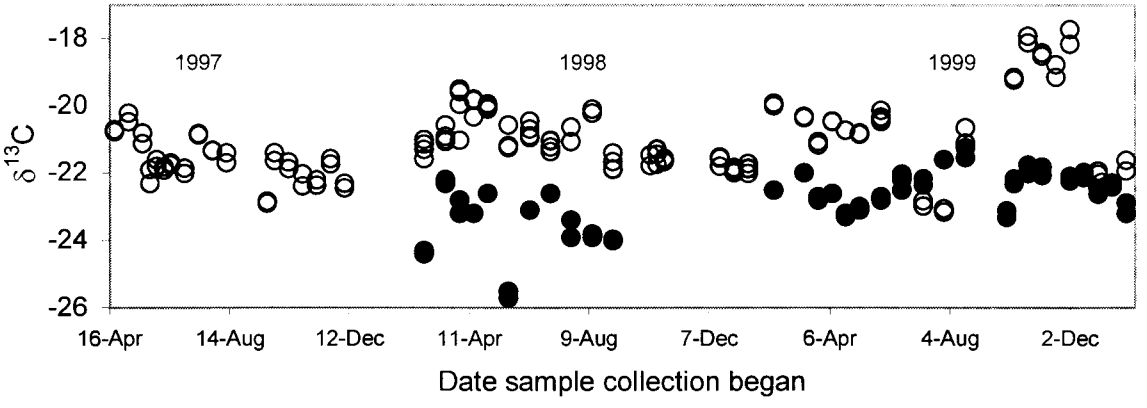

Figure 2.5. $\delta^{13}C$ (‰) of sediment trap samples collected at the middle shelf site M2 (o) and the outer shelf site M3 (•), April 1997 through January 2000. No samples were collected at M3 during 1997. Multiple symbols at a single time represent replicate analyses of a single sediment trap sample.

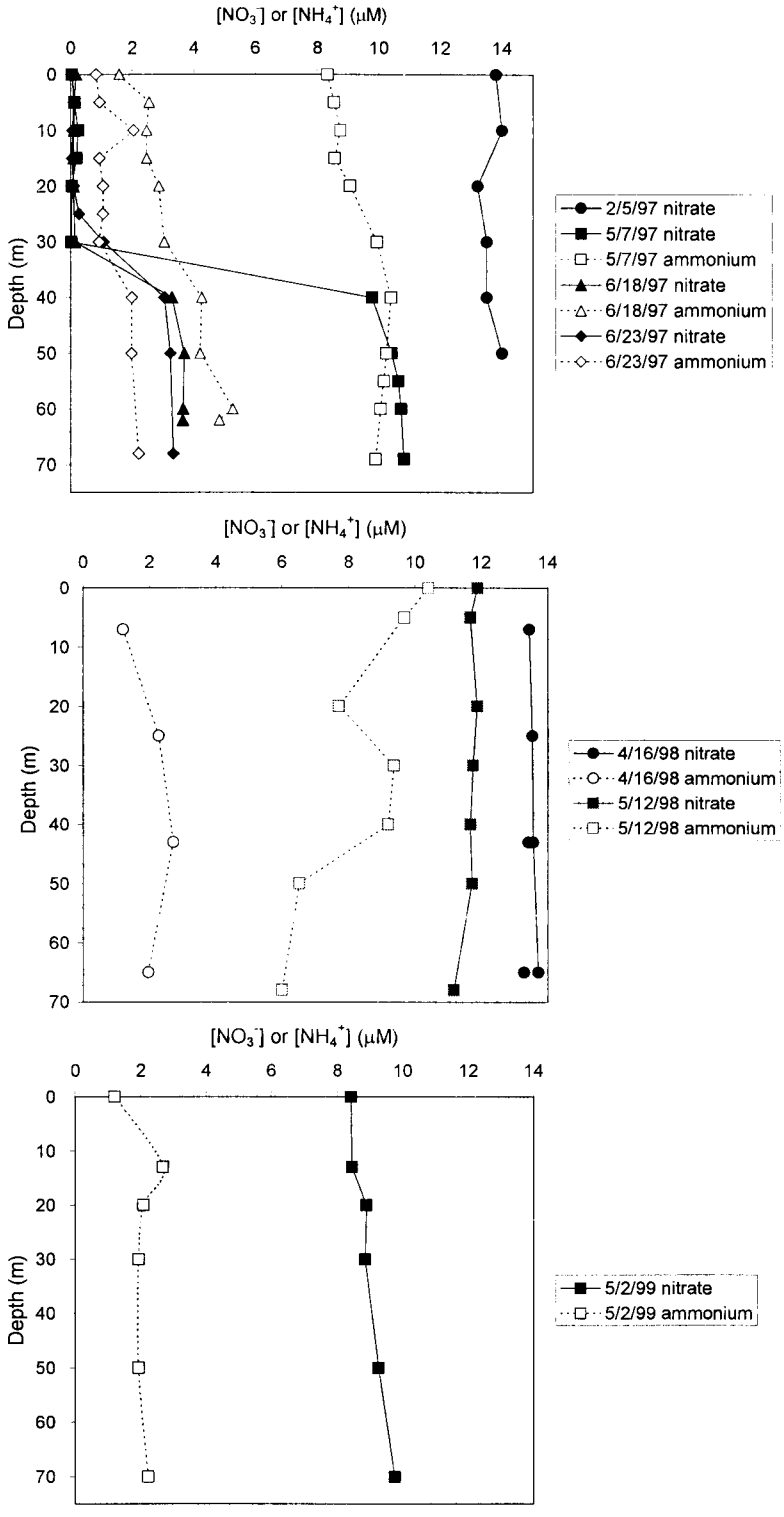

Figure 2.6. Nitrate and ammonium concentrations at middle shelf site M2, 1997-1999. Data are courtesy of Dr. Terry Whitledge, University of Alaska Fairbanks.

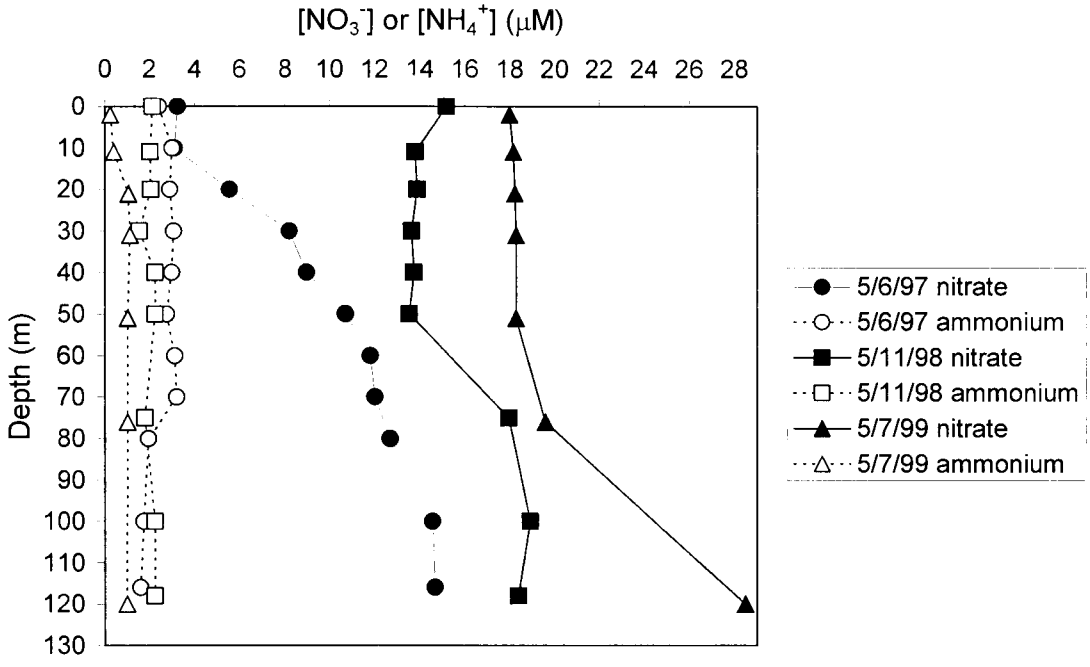

Figure 2.7. Nitrate and ammonium concentrations at outer shelf site M3, 1997-1999. Data are courtesy of Dr. Terry Whitledge, University of Alaska Fairbanks.

Table 2.1. Stable isotopic composition of zooplankton over the southeastern Bering Sea shelf, 1997-1999.

Year Collected	Zooplankton type	Station	Mean 1[a] $\delta^{15}N$ (‰)	s.d.	Mean 1[a] $\delta^{13}C$ (‰)	s.d.	Mean 2[a] $\delta^{15}N$ (‰)	Mean 2[a] $\delta^{13}C$ (‰)	n
1997	Copepod	M2	13.2	1.5	-22.8	1.8	13.6	-22.4	7
1997	Copepod	M3	9.0	1.2	-24.8	3.8			5
1998	Copepod	M2	9.8	1.0	-23.2	0.7	9.9	-22.8	15
1998	Copepod	M3	7.5	1.5	-25.1	1.9			14
1999	Copepod	M2	10.7	1.0	-20.4	0.9	10.8	-20.6	9
1999	Copepod	M3	7.9	1.1	-25.1	1.5			21
1997	Euphausiid	M2	12.3	1.7	-20.0	1.9	12.5	-19.8	7
1997	Euphausiid	M3	9.6	0.2	-20.2	0.2			5
1998	Euphausiid	M2	10.4	1.4	-20.7	0.8	10.8	-20.5	16
1998	Euphausiid	M3	8.6	1.3	-21.8	1.1			10
1999	Euphausiid	M2	10.0	2.5	-19.0	1.1	11.2	-19.0	12
1999	Euphausiid	M3	9.3	0.9	-22.4	0.6			9
1997	Chaetognath	M2	15.2	1.1	-20.7	0.8			8
1997	Chaetognath	M3	12.9		-21.8				1
1998	Chaetognath	M2	15.0	1.4	-21.8	0.6			17
1998	Chaetognath	M3	12.6	1.6	-22.1	1.6			7
1999	Chaetognath	M2	14.6	1.2	-21.1	0.7			12
1999	Chaetognath	M3	12.6	0.8	-22.4	0.9			9
1997	Scyphozoan	M2	14.8	2.1	-20.5	0.3			7
1997	Scyphozoan	M3	10.3	0.8	-22.5	1.5			2
1998	Scyphozoan	M2	12.6	0.8	-20.1	0.9			10
1998	Scyphozoan	M3	11.0	1.2	-21.7	0.8			5
1999	Scyphozoan	M2	13.0	1.1	-19.7	0.7			8
1999	Scyphozoan	M3	10.8		-20.6				1

[a]Mean 1 is the simple average of all data for this zooplankton type at the given site and year. Mean 2 was calculated by first averaging the data for each month sampled, and then averaging those means to obtain an annual mean. September, 1999 data were omitted from Mean 2, since September samples were collected only in 1999.

Table 2.2. Stable isotopic composition of suspended particulate matter (SPOM) and net plankton (NP) over the southeastern Bering Sea shelf, 1997-1999.

Sampling date	Sample type	Station	$\delta^{15}N$ (‰)	s.d.	$\delta^{13}C$ (‰)	s.d.	n
Jun-97	NP	m2	12.6	1.2	-24.8	0.0	2
		m4	13.2	1.7	-23.2	0.2	3
Apr-98	NP	m2	11.4	0.7	-21.8	1.3	2
	NP	m4	12.3		-20.1		1
May-98	NP	m2	7.1		-20.6		1
Apr-98	SPOM	m2	13.6	1.9	-21.2	0.9	12
Apr-98	SPOM	m2	13.1	1.3	-21.6	0.9	3
May-98	SPOM	m2	10.4	2.2	-21.4	0.1	3
May-98	SPOM	m2	15.1	2.2	-21.5	0.5	6
May-98	SPOM	m2	12.3	1.0	-21.6	1.1	3
May-98	SPOM	m2	12.4	2.0	-21.9	0.3	3
Mean 1998	SPOM	m2	13.3	2.2	-21.4	0.7	30
May-98	SPOM	m3	10.5	1.0	-25.5	0.2	3
May-98	SPOM	m3	10.0	2.7	-24.4	0.3	3
May-98	SPOM	m3	12.7	3.0	-26.2	0.1	3
Mean 1998	SPOM	m3	11.1	2.4	-25.4	0.8	9
May-99	NP	m2	6.6	0.1	-18.2	1.4	3
	NP	m4	8.0	1.1	-19.7	1.1	3
May-99	SPOM	m2	9.1		-24.1		1
May-99	SPOM	m2	6.9	0.5	-19.8	0.1	2
May-99	NP	m3	6.7	1.2	-26.5	0.6	3
May-99	SPOM	m3	6.2		-25.8		1

Table 2.3. Weighted mean of sediment trap $\delta^{15}N$ and $\delta^{13}C$ for spring and summer periods. The isotopic data were weighted by the quantity of organic carbon collected by the trap during each sampling period.

Station	Sampling period[a]	Mean $\delta^{15}N$ (‰)	Mean $\delta^{13}C$ (‰)
M2	4/22/97-5/27/97	13.0	-20.8
M2	4/22/97-8/12/97	13.6	-21.1
M2	4/2/98-5/21/98	13.9	-20.1
M2	4/2/98-8/13/98	13.8	-20.3
M2	4/9/99-5/7/99	10.4	-20.6
M2	4/9/99-8/20/99	9.7	-20.9
M3	4/2/98-5/21/98	10.9	-23.0
M3	4/2/98-9/2/98	10.8	-22.9
M3	4/9/99-5/7/99	9.6	-23.0
M3	4/9/99-8/20/99	9.4	-22.3

[a]The dates sediment trap sample collection began, i.e., the last sample was collected for 1-2 weeks after the final date shown.

Chapter 3.

Variability in the composition and quantity of fatty acids and sterols in sinking particles at two sites over the southeastern Bering Sea shelf*

Key Words: Lipids, biomarkers, fatty acids, sterols, sediment traps, phytoplankton, zooplankton, fecal pellets, Alaska, Bering Sea

Abstract

Fatty acid and sterol composition were measured in particulate organic matter collected using sediment traps moored at two sites over the Bering Sea shelf from February 1998 through February 2000. Middle shelf (M2) sediment trap samples contained higher concentrations of fatty acids typical of diatoms, such as the fatty acid 16:1ω7, while outer shelf (M3) samples contained more saturated fatty acids typical of zooplankton. Phytoplankton counts of trap subsamples showed that intact diatoms were often 20 times more numerous in the M2 trap than the M3 trap. The dominant PUFA (polyunsaturated fatty acid) in both middle and outer shelf samples was 20:5ω3, which is often a significant component of both phytoplankton and zooplankton phospholipids. Other PUFA included 20:4ω6, 22:5ω3, and 22:6ω3. Neutral lipid composition of particulate matter was comparable at the two sites, with cholesterol being the dominant sterol. Other sterols included 24-norcholesta-5,22E-dien-3β-ol, cholesta-5,22E-dien-3β-ol and 24-methylcholesta-5,22E-dien-3β-ol. Sterols were a greater proportion of

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neutrals at M2 during late winter-early summer, usually over 60%. The fatty alcohol 20:1ω9 was the major neutral during the remainder of the year. Sterols contributed the greatest proportion of neutrals at M3 throughout the year, except during the spring bloom, when the fatty alcohols 16:0, phytol, and especially 20:1ω9 and 22:1ω11 (over 66% of the total) were dominant. Small fluxes of stanols (saturated sterols) were also found. Diacylglyceryl ethers (DAGE), from diatoms and from the pteropod *Clione limacina*, and trace amounts of steroid ketones (intermediate compounds in sterol to stanol transformation) were also found among the neutrals.

3.1. Introduction

The southeastern Bering Sea shelf is an economically and ecologically important ecosystem, subject to substantial natural and human-induced change (Paine *et al.*, 2003). Since the Bering Sea shelf is a high latitude ecosystem, primary productivity is highly dependent on seasonal light and temperature changes. In addition to the seasonal changes, variability in weather and climate also affects primary production and its coupling to higher trophic levels (Hunt *et al.,* 2002). The middle and outer shelf regions differ in their plankton ecology. Middle shelf phytoplankton-zooplankton blooms are often uncoupled, but outer shelf blooms are usually coupled. Phytoplankton blooms of chain-forming diatoms such as *Rhizosolenia spp.* often remain ungrazed by the middle shelf's smaller copepods (Alexander and Cooney, 1979). Larger copepods on the outer shelf usually graze those blooms efficiently.

Diatoms are major primary producers in the Bering Sea ecosystem. Since 1997, the prymnesiophyte *Emiliania huxleyi* has also become dominant during blooms that occur mainly in fall and cover large areas of the middle and outer shelf (Stabeno and Hunt, 2002). The Bering Sea food web is unusually productive at higher trophic levels, including abundant sea birds, pelagic fishes, whales and pinnipeds. In addition, phytoplankton and zooplankton supply sinking particulate matter to sediments, supporting highly productive benthic communities.

Tracer compounds such as lipids can be used to decipher spatial and inter-annual variations in sources of organic matter (Falk-Petersen *et al.*, 1998; Henderson *et al.*, 1998; Scott *et al.*, 1999). These tracers give clues to nutritive values of food

particles (Cotonnec *et al.*, 2001) and food web interactions (Neal *et al.*, 1986); they also may serve as chemotaxonomic markers, enabling identification of organic matter sources (Volkman *et al.*, 1993; Mansour *et al.*, 1999). Wakeham *et al.* (1997) have utilized fatty acid and sterol tracers in sediment trap organic matter as means of pinpointing organic matter sources, elucidating diagenetic changes in particulate matter through the water column and examining seasonal, inter-annual and spatial variability in fluxes. Fatty acids and other lipid classes were used by Conte (1990) as tracers of transport and transformation pathways to investigate the particulate organic carbon cycle in warm-core rings. The neutral lipid composition of diatoms has been used to differentiate species (Nichols *et al.*, 1983; Volkman *et al.* 1998).

This study describes patterns and variations in sinking particulate matter quantity and quality over the Bering Sea shelf at the two sites, using the lipid content of sediment trap samples. Use of time-series sediment traps allows for the investigation of shelf particle dynamics year-round. This research was part of the SEBSCC (Southeast Bering Sea Carrying Capacity) program; the aim of SEBSCC was to assess the state of the ecosystem and its carrying capacity for walleye pollock (Macklin *et al.*, 2002).

3.2. Study Area and Plankton Ecology

The study area was located on the southeastern Bering Sea shelf (Figure 1.1). The shelf is broad, 500 km wide, and is commonly divided into three distinct hydrographic regions (the inner, middle and outer shelves), which are separated from

each other by density fronts (Iverson *et al.*, 1979; Kinder and Schumacher, 1981; Coachman, 1986). The regions differ in water column depth, depth profiles of temperature, salinity and nutrient concentration, and the plankton and animal species at higher trophic levels (Kinder and Schumacher, 1981; Coachman, 1986). This sediment trap study was conducted at a middle shelf station and an outer shelf station. One sediment trap, current meters, and instruments measuring temperature, salinity and chlorophyll (Stabeno *et al.*, 2002) were moored at each site. The middle shelf (situated between the 50 m and 100 m isobaths) is often ice-covered during cold winters and ice-free during mild winters. Increased solar radiation, warm temperatures, and water column stability trigger an ice-edge phytoplankton bloom as ice recedes in mid- to late-April (Niebauer *et al.*, 1995). Open water blooms during warm years usually begin in May. Diatoms such as *Coscinodiscus* spp., *Thalassiosira* spp. and *Chaetoceros* spp. are primary producers in middle shelf waters (Allen, 1927; Kawarada and Ohwada, 1957; Alexander and Cooney, 1979; Schandelmeier and Alexander, 1981). Coccolithophorids and dinoflagellates also are found over the middle shelf (Alexander and Cooney, 1979; Napp and Hunt, 2001).

Smaller calanoid copepods, such as *Pseudocalanus* spp. and *Calanus marshallae*, and the euphausiid *Thysanoessa raschi,* which can comprise an average of 88% of the zooplankton biomass in April (Smith, 1991), are typical consumers over the middle shelf. The zooplankton biomass is usually low in early spring at the time of the phytoplankton bloom, although euphausiids may be abundant; copepod development occurs in May through June, and euphausiids spawn in May, possibly following the

seasonal progression of temperature (Vidal and Smith, 1986). Therefore, the bloom is often not grazed extensively (Cooney and Coyle, 1982). The outer shelf rarely has sea ice or ice-edge blooms. An open water mixed diatom bloom generally occurs there, although *Chaetoceros* spp. often dominated the phytoplankton biomass during 1997-2001 (Smith and Henrichs, unpublished data). Larger, oceanic copepods such as *Neocalanus cristatus* and *Neocalanus plumchrus* and the euphausiid *Thysanoessa inermis*, which spawns in early April (Vidal and Smith, 1986), live in outer shelf waters. These zooplankton over-winter as adults and are numerous during the spring phytoplankton bloom.

3.3. Methods

3.3.1. IRSC sediment traps

Two indented rotating sphere sediment traps (Figure 1.2), equipped with an eleven-sample carousel, collected a time series of sinking particles (Peterson *et al.*, 1993). A feature of this trap design is the indented rotating sphere, the function of which is to exclude swimmers from sample tubes. Most samples were swimmer-free. Occasionally, one or two small copepods were found and picked out of samples. In a single event, a large number of pteropods were deposited into the September,1999, sample at M2. The traps were moored at two sites over the Bering Sea shelf. Trap M2 was deployed over the middle shelf (56°53'N, 164°02'W) at 35 meters, where the water depth was 70 m (Figure 1.1). Trap M3 was moored at 70 meters at an outer shelf site (56°04'N, 166°20'W). The water depth at this site was 120 m. Trap M2 has been

deployed year-round since April 1997. Trap M3 was deployed February through September 1998, and year-round in 1999. The traps were turned around twice a year, during a winter cruise in February and during a fall cruise in September. Prior to trap deployments 5 g NaCl and 50 mg $HgCl_2$ were placed in sample tubes to retard bacterial activity. Sample collection intervals were two or three weeks, depending upon projected influx of material to the traps. Upon retrieval, samples were stored in pre-combusted sample jars and frozen until split for analysis.

3.3.2. Microscopy

A Zeiss Telaval 31 inverted microscope was used to count and identify phytoplankton in trap samples. Ten ml of each subsample was preserved in 5% formalin. An aliquot of each subsample was thoroughly mixed, dispersed in a 25-ml capacity plate-settling chamber, and allowed to settle for 24 hours. Phytoplankton that settled on the plate were counted up to a total of 300 cells. Whenever possible, phytoplankton were identified to species. Other particulate material, such as skeletal remains, tests and fecal pellets (zooplankton and dinoflagellate) were noted.

3.3.3. Lipids

Lipids were extracted from sediment trap samples using Soxhlet extractors and a solvent system of 2:1 CH_2Cl_2:MeOH (Wakeham *et al.*, 1997). Sediments were extracted for 24 hours. Following extraction, a 5% NaCl solution was added. The solvents and water were transferred to separatory funnels, and the lower, organic phase was separated and filtered using a 0.45 μm Teflon® filter. The filtered solvent was placed in a 50 ml Teflon® test tube and evaporated to dryness using a centrifugal

evaporator. Two ml of 1:1 hexane:$CHCl_3$ were added and the sample was stored at -40° C until saponification. A 100-µl or 200-µl aliquot (depending upon the amount of material) of each total solvent extract was transferred to a pre-combusted test tube. The total solvent extract (TSE) was evaporated to dryness, then saponified using 2 ml 5% w/v KOH in 80/20 methanol/glass distilled water and heated at 60° C for 3 hours. One ml of water was added, and then the neutral lipid fraction was extracted from the TSE using 4:1 hexane:$CHCl_3$. Recovered neutrals were derivatized using 50 µl BSTFA to form TMS esters. Samples were made up to a volume of 2 ml. Cholestane was used as an internal GC standard. The TSE was acidified with 0.3 ml of HCl and fatty acids were extracted with 4:1 hexane:chloroform. Acid fractions were methylated with 3 ml 10:1:1 CH_3OH:HCl: $CHCl_3$ and heated for 1 hour at 100° C. One ml glass distilled water was added, and the fatty acid methyl esters were extracted three times with the hexane solvent. Solutions were then made up to 2 ml volume, and methylnonadecanoate was added as an internal standard.

3.3.4. Gas chromatography

TMS derivatives and fatty acid methyl esters were analysed by GC-MS using a Hewlett-Packard 5890 Series II Plus Gas Chromatograph. A 30 m X 0.25mm inner diameter capillary column with splitless injection was used with helium as the carrier gas. Injection temperature was 250° C and detector transfer line temperature was 300° C for fatty acids. Initial oven temperature was held at 180° C for two minutes then ramped at a rate of 2° C/min to 250° C. The flow rate was 1 ml/min. For sterol and fatty alcohol derivatives, injection temperature was 250° C and detector temperature

was 290° C. Initial oven temperature was held at 180° C for four minutes, and then ramped at 3° C/min to 310° C.

3.3.5. Data analysis

Data were analyzed statistically using SPSS (Graduate Pack 11.0) software. Principal components analysis (PCA) was used to extract the subsets of lipids influencing each sampling season. PCA reduces a large number of variables into a smaller number of subsets, called components. It also extracts information about major influences on a data set. For instance, in this data set, a dominant fatty acid would have a greater loading on one of the components. The analysis was performed on variables representing one year's worth of data. Four PCA analyses were done for this study, one for each year (1998 and 1999) at the two sites. PCA was attempted on seasonal data sets instead of annual data sets, but in most instances, these failed to produce significant relationships. Once the components were extracted, the results were "rotated" to enhance interpretability of the solution. Rotating the data magnifies important factors in the data set and minimizes those which are less important. An oblique rotation, Promax, was enlisted, using a kappa of 4.

3.4. Results

3.4.1. Pooling of data

To simplify data analysis, sampling interval data were pooled into a sampling period based on similarity of lipid content and phytoplankton content of intervals. For instance, sampling intervals one through five, M2 1998, all contained similar relative

concentrations of major fatty acids, sterols and phytoplankton, and similar quanitities of lipids collected.

3.4.2. 1998

The quantity of lipids collected by the M2 sediment trap between 2/98 and 5/98 was the highest of all years and sites studied (Figure 3.1a, 3.2.a). The average daily collection between February and May was 44 mmol lipid-C m^{-2} day^{-1}. This lipid quantity was over 100 times greater than that collected at M3 during the same time interval (Figure 3.1a). In early 1998, neutral lipids made up more than 53% of the lipids at M2, with cholest-5-en-3β-ol (cholesterol) and cholesta-5,22E-dien-3β-ol, considered animal-derived sterols, accounting for 36% and 7% respectively. Only traces of neutral compounds were found in M3 trap samples. The M3 trap samples from mid-June through September were composed only of small, unidentified fecal pellets. The lipid content was only a trace amount. The diatom indicator, 16:1ω7, was a greater percentage of the lipids in M2 traps than in those from M3 (Figure 3.1b). Corresponding to this, diatoms represented a larger fraction of the phytoplankton in M2 samples (Figure 3.1c). Phytoplankton counts of subsamples showed diatoms were between 8 and 48 times more numerous than at M3 during 1998.

3.4.3. 1999

The quantity of lipids collected in M2 and M3 trap samples was similar throughout the year except during the four-month interval spanning 7/99-11/99. The lipid amount collected at M3 was two to three times greater, due to an increase in polyunsaturated fatty acids (PUFA) and especially in sterol concentration (Figure

3.2a). The largest amount of lipid collected at M2 was in early spring (2/99-5/99). Saturated fatty acids (29.3%) such as 18:0, and cholesterol (21.8%) were the dominant compounds. At M3, cholesterol was a larger portion of the lipids, increasing seasonally from late winter 1999 through early winter 2000. Saturated fatty acids exhibited the opposite trend (Figure 3.3). Similar to 1998 trap samples, the diatom indicator 16:1ω7 was always a greater percentage of the total lipid at M2 than at M3 (Figure 3.2b). The fatty acid was never greater than about 6% of the total lipids at M3, but was nearly 30% of total lipid at M2 after the May ice retreat. Diatom counts (Figure 3.2c) of trap samples revealed that diatoms made up as much as three-quarters of M2 sample counts, but that diatoms were rarely even one-third of the total phytoplankton in M3 sample counts. Pooled diatom counts from both sites and years showed a high correlation (r^2=0.81) between the number of diatoms counted in trap samples and 16:1ω7/total lipid (Figure 4). Coccolithophorids bloomed in the surface water over both sites in fall 1999. The highest concentration of the prymnesiophytes in trap samples was found from 9/99 to 11/99 at both sites.

3.5. Discussion

3.5.1. Quantity of lipid collected

The quantities of lipids collected by the sediment traps are reported in this paper rather than the lipid fluxes. Sediment traps often do not collect sediment particles with 100% efficiency (Buessler, 1991; Cochran *et al.*, 1993). Tidal currents and resuspended bottom sediment, especially over shallow sites such as M2 and M3,

can affect quantitative particle collection. Smith *et al.* (2002) fully addresses these topics in relation to the Bering Sea sediment traps.

3.5.2. Principal components analysis

In order to reduce the complexity of the data sets and to discover which fatty acids and sterols heavily influenced the results, PCA was used. In PCA once the analysis has been performed and the components extracted, each variable is assigned a coefficient or "loading". Loadings represent a variable's influence on a component; high loadings, those with absolute values closer to 1, have a greater impact on that component, while loadings near 0 have the least impact on the component. Promax rotation increases interpretability of the output by minimizing small loadings to nearly zero and reducing large loadings also, but not to zero. A common practice is to plot loadings from the first component (responsible for the greatest percentage of variability within the data set) against loadings from the second component.

In Figure 3.5, component loadings were plotted, displaying influential variables and showing groups of variables. Data from M3 1998 are graphed in Figure 3.5a. The fatty acids are loosely gathered into three groups. The two groups influencing component 1 the most were the saturates 14:0 and 16:0, at a loading of around –1, and the 20 PUFA and monounsaturated fatty acids (MUFA), at a loading of about 1, plus 18:0 and 18:1ω9. The factors which could influence the grouping of these variables are more degraded material (saturates) versus more labile, fresher material with a planktonic source (MUFA and PUFA).

The MUFA (16:1ω7, 18:1ω7 and) have high positive loadings on component

2, while the C_{20} PUFA have high negative loadings. This may indicate differences in fatty acid sources, for example algal or herbivore (MUFA) as opposed to omnivore or carnivore (PUFA). The loadings could also point to a difference in the lipid sources of the fatty acids. Monounsaturated fatty acids tend to originate in diatoms but are often used by herbivores in their neutral lipids, such as wax esters and triacylglyercols. Conversely, organisms use polyunsaturated fatty acids as part of their polar lipids in cellular membranes. The data show a sparse input of fatty acids and neutral lipids at M3 in 1998. Greater coupling between zooplankton and phytoplankton could be responsible for the paucity of material. Since zooplankton fecal pellet input is important to the sinking particle flux at M3, it is possible that zooplankton were being consumed by predators in 1998. Another factor affecting the flux of large sinking particles to depth is coprophagy. Saturated fatty acids are major components of fecal material produced by coprophagy (Harvey *et al.*, 1987)

Two lipid groups influence the principal components in Figure 3.5b. Neutral lipids, such as C_{27} and C_{28} sterols and 1-O-alkyl glycerol ethers, have high loadings on principal component 1. These lipids may represent input from fecal pellets containing more polar lipids, or lipids that had a high concentration in sediment trap material in fall. Diatom fatty acids and herbivorous zooplankton fatty acids and fatty alcohols have high loadings on PC2. Simply, high loadings on PC1 show input from feeding zooplankton; PC2 shows diatom and calanoid copepod input. Since 1998 was an unusally warm year, and the phytoplankton and zooplankton lipids were highly related, the data support the notion of greater coupling of primary and secondary production

during warm years.

Figures 3.5c and 3.5d represent M3 1999 and M2 1999, respectively. In Figure 3.5c, neutral lipids have high positive loadings on principal component 1 (which accounts for 37.6% of the variance). The neutrals indicate input from zooplankton fecal material due to cholesterol, other C_{27} sterols and input from C_{28} sterols, which originate from diatoms. These C_{28} sterols were most likely excreted with zooplankton fecal pellets. On PC2 (30.8% of the variance), high positive loadings for monounsaturated fatty acids and fatty alcohols represent input from herbivorous zooplankton. Principal components analysis of M3 1999 data suggests that zooplankton input dominated sediment trap material. Predators grazing on zooplankton probably decreased from 1998. Also, coprophagy was not as significant. In Figure 3.5d, monounsaturates, diatom indicators such as 14:0, 16:0, and 16:1ω7 and C_{18} PUFA are loosely grouped together on PC 1, showing a common source, probably diatoms. The positive loadings on PC 1 include the neutrals and C_{20} PUFA and C_{22} PUFA. Since diatom indicators 16:1ω7 and 16:0 have high loadings on PC1, the influence of diatoms was high. This suggests that some of the phytoplankton bloom went ungrazed. Zooplankton neutral lipids also had high loadings on PC1. This indicates that zooplankton also influenced sinking particles. The major diatom input was during spring, and zooplankton input was greater in fall.

3.5.3. 1998

Unusual conditions such as high heat content of the water and warmer-than-average sea surface temperatures (Stabeno and Hunt, 2002) may be indirectly

responsible for large lipid fluxes to the 1998 M2 trap, in which cholesterol was dominant. Rho (2000) found abnormally high ammonium concentrations in spring 1998, possibly due to increased zooplankton excretion. Napp *et al.* (2002) found that spring 1998 *Calanus marshallae* numbers were increased significantly over spring 1997 numbers. However, zooplankton collected at M2 in February, April and May 1998 contained little or no cholesterol. This is consistent with other reports. For instance, Scott *et al.* (1999) reported *Calanus hyperboreus* specimens collected from the marginal ice zone of the Barents Sea as being just 1.1% (±1.4) cholesterol (as % of total lipids). In a study conducted on Antarctic zooplankton (two calanoid copepods and two euphausiids), Falk-Petersen *et al.* (1999) found sterols were less than 10% of total zooplankton lipids. Calanoid copepods cannot synthesize sterols *de novo* (Goad, 1981) and therefore must obtain them from their diet.

So, why does cholesterol predominate among neutral lipids in sediment traps? Although some diatoms are known to possess cholesterol as their major sterol (Tsitsa-Tzardis *et al.*, 1993), in general cholesterol is not a dominant lipid in diatoms. Phytoplankton collected at M2 contained cholesterol (Smith and Henrichs, unpublished data), but not as the dominant lipid. Zooplankton excrete excess cholesterol and dealkylated phytosterols, thereby increasing the sterol content in fecal pellets (Volkman *et al.*, 1980; Prahl *et al.*, 1984; Harvey *et al.*, 1987). Coprophagy and carnivory increase fecal pellet cholesterol over herbivory (Prahl *et al.*, 1984; Prahl *et al.*, 1985). Therefore, it is concluded that zooplankton fecal pellets were the source of cholesterol.

The same unusual oceanic conditions may have affected M3 differently in 1998, which had the smallest quantity of lipids collected over the duration of the study, and, in general, contained very little material overall. A few fecal pellets, marine snow and detritus were present. The sediment trap contained a computer that recorded information on the trap's operation. No malfunctions were found in the log. Also, no obstructions were found at the mouth of the trap when it was retrieved. Therefore, it is concluded that the trap functioned properly in 1998, despite the small amount of material collected. Contrary to the small amount of particles collected, the depth averaged primary productivity and the f-ratio were greater at M3 than at M2 during May (Rho, 2000). More tightly coupled phytoplankton-zooplankton blooms and zooplankton grazing pressure likely decreased the flux of diatoms to depth at M3, as evidenced by few diatoms in the M3 samples and the low concentration of 16:1ω7. The fact that the M3 trap (70 m) was moored twice as deeply as the M2 trap (35 m) is probably not important, since the sinking rate of large particles (i.e., fecal pellets) is hundreds of meters per day (Bishop *et al.*, 1977).

Other factors may account for the low lipid content and in particular, low PUFA, in the trap. Sea surface temperatures and the water column heat content were well above average. Nutrients were abundant in surface waters, at least through May. Under these conditions, phytoplankton and zooplankton would not produce lipids synthesized under more extreme conditions, such as nutrient limitation or low sea surface temperatures (Zhukova and Aizdaicher, 2001). Another factor could be that phytoplankton lipids, and PUFA specifically, were either quickly utilized by

zooplankton grazers or microbially oxidized within the euphotic zone. Rho (2000) speculated that remineralization of organic matter was speeded up in 1998 due to warmer water column temperatures. It has been found that coprophagy essentially eliminates PUFA from fecal pellets (Neal *et al.*, 1986) and leaves predominately saturated fatty acids behind. Only trace amounts of lipids that zooplankton excrete, such as cholesterol, were found. This may indicate that grazing pressure on zooplankton was increased in 1998.

In addition, Stabeno *et al.* (1999) reported a shift in shelf circulation patterns in 1998. Water from the shelf break and outer shelf was advected landward, importing oceanic zooplankton species to the inner shelf area (Coyle and Pinchuk, 2002a,b). Grazing pressure may have depleted zooplankton numbers on the outer shelf. Also warmer temperatures accelerated zooplankton development, possibly resulting in early ontogenetic migration to depth. This may explain why the flux of carbon decreased in the fall, although a diatom bloom occurred at M2, as indicated by phytoplankton observed in the trap sample. *Neocalanus plumchrus*, which dominates the zooplankton biomass throughout the outer domain (Vidal and Smith, 1986), was found to have decreased significantly in biomass in May 1998 compared with May 1980 and May 1996 (Napp *et al.*, 2002).

3.5.4. 1999

The quantity of lipid collected by the M2 trap was greater than that collected at M3 only during one sampling interval, 2/99-5/99. The retreat of the ice edge in March caused a diatom bloom at M2 and the subsequent deposition of the plant lipids 16:1ω7

(10% of total lipids) and 20:5ω3 (11%). Lipids concentrated in zooplankton fecal pellets, such as cholesterol (22%) and the saturated fatty acid 18:0 (15%) (Neal *et al.*, 1986; Harvey *et al.*, 1987) were also abundant in the sample. Omnivorous zooplankton were the most likely source of the lipids. Fatty alcohols, indicative of zooplankton wax esters, were not found in M2 trap samples until July. In most instances, herbivorous calanoid copepods do not obtain fatty alcohols from their diet but biosynthesize them from dietary fatty acids and from *de novo* synthesized fatty acids (Sargent and Henderson, 1986). Some phytoplankton have been found to possess wax esters as a small percentage of their lipids.

Only trace amounts of sterols were found in the samples from 5/99-7/99, which seems to indicate that the collected material was mainly composed of sinking diatoms rather than fecal pellets. The fatty acids 16:1ω7 and 20:5ω3, in addition to 14:0 and 16:0, have been found to dominate the diatom fatty acid profiles (Dunstan *et al.*, 1994; Henderson *et al.*, 1998; Falk-Petersen *et al.*, 1998). This relates to the cold temperatures in 1999, low zooplankton abundance and low production and grazing (Coyle and Pinchuk, 2002b).

A more diatom-like lipid signature was present at M2 than at M3, which is evident from Figures 3.1c and 3.2c. In addition, 16:1ω7 and 20:5ω3 were negatively correlated at M2. According to Zhukova and Aizdaicher (2001), who conducted a study on diatom lipid content during vegetative and resting stages, the polar lipid and PUFA content increases under unfavorable environmental conditions, such as low sea surface temperatures or nutrient depletion. During more favorable conditions, diatoms

accumulate storage lipids, which are high in saturated and monounsaturated fatty acids. This is consistent with the M2 fatty acid profile, although nutrient limitation and not sea surface temperature seems to have driven the seasonal lipid production. For example, during diatom production, the monounsaturate 16:1ω7 dominated the fatty acids during the March pulse and in May-early July (29%). From July through mid-September, PUFA, especially 20:5ω3, 22:5ω3 and 22:6ω3 (24%), were the major fatty acids. The average sea surface temperature from 7/99-9/99 was about 5°C warmer than during the May-July period, but nutrients were depleted in the sea surface and diatom production slowed (See Chapter 2). During the coccolithophorid bloom and further nutrient depletion, the PUFA concentration increased further to 35%. The fatty acid signature is consistent with that expected from the low nutrient conditions observed at M2 during the summer-fall 1999.

Zooplankton inputs dominated the sediment trap samples from M3, consistent with knowledge of the pelagic food web at this site. First, the biomass of calanoid copepods is greater at site M3 than M2, due to the large oceanic species present there. Therefore, it would be expected that a larger proportion of the lipids would be derived from zooplankton. The saturated fatty acid 18:0 was on average the dominant fatty acid from 2/99-9/99. Cholesterol was the dominant lipid from 7/99-1/00. Both of these have been shown to be concentrated in calanoid copepod fecal pellets (Harvey *et al.*, 1987). Saturated fatty acids (mainly 18:0) and cholesterol were negatively correlated ($r=-0.94$; $r^2=0.88$). This was consistent with results of a feeding experiment (Harvey *et al.*, 1987), which showed that the relative concentration of 18:0 in copepod

fecal pellets decreased as food supply increased. They also found that the relative concentration of cholesterol in fecal pellets decreased compared to other sterols as the feeding level increased, but that the total amount of sterols increased commensurate with the increase in dietary sterols.

A massive coccolithophorid bloom covered the outer shelf beginning in August 1999, which likely resulted in increased food supply for zooplankton at both M2 and M3. According to Olson and Strom (2002), microzooplankton grazed the coccolithophorid bloom while macrozooplankton grazed on the microzooplankton. Cholesterol decreased from 94% of the total sterols (2/99-5/99) to 63.5% of the total (9/99-11/99). Cholesterol can have both zooplanktonic and phytoplanktonic sources (Volkman, 1986; Tsitsa-Tzardis *et al.* 1993;Barrett *et al.*, 1995). Other sterols that increased in concentration in September samples included 27-nor-24-methylcholesta-5,22-dien-3β-ol (10.7%), 24-norcholesta-5,22-dien-3β-ol (4.7%) and cholesta-5,22-dien-3β-ol (5.9%), all present in zooplankton fecal pellets although not necessary in the algal diet. Plant-derived sterols such as 24-methylcholesta-5,22-dien-3β-ol (8.1%) and 24-methylcholesta-5,24(28)-dien-3β-ol (2.6%) were also present. Both 24-methyl sterols are considered diatom indicators, although 24-methylcholesta-5,22-dien-3β-ol (once dubbed diatomsterol) is now thought to be ubiquitous among phytoplankton (Barrett *et al.*, 1995). This indicates that zooplankton were feeding on diatoms during fall, not only in the spring.

Fatty acids, such as the diatom indicator 16:ω7, were only a small portion of the

total lipids at M3. PUFA increased from 7/99-11/99. This trend is opposite to that reported in most fecal pellet production experiments. In those cases, most PUFA were quickly utilized by the zooplankton. One explanation could be that the PUFA were present in phytoplankton descending to the trap through mixing. Many trap samples contained input from diacylglyceryl ethers or 1-O-alkylglyerol ethers, a storage lipid found in high concentrations in squid and sharks. They consist of a glycerol backbone with acyl groups attached at the 2 and 3 positions and an alkyl group attached at the 1 position. The pteropod *Clione limacina* was the only zooplankter collected that possessed this lipid as its major neutral lipid. The only two alkyl groups found atttached to the glycerol backbone in Clione were 15:0 and 16:0. The alkyl groups found in sediment trap samples were 16:0, 18:0, 18:1 isomers and 20:1 isomers. Clione's predators may metabolize the DAGEs, utilizing the acyl groups (which are predominantly 15:0, 15:1, 17:0, 17:1 (two isomers) and 19:1 (two isomers).

3.6. Conclusions

Weather over the southeast Bering Sea affects primary and secondary production. A comparison between a warm year (1998) and a cold year with sea ice in spring (1999) showed that weather affects the quantity and the quality of material that settles to the sea floor. The weather signal can be seen across the shelf at both middle and outer shelf sites. However, site M2 and site M3 responded differently to environmental forcings. The following can be concluded:

- Fatty acids indicative of diatoms had higher concentrations in M2 samples.

- The flux of diatoms to the M2 trap was greater than that to the M3 trap, regardless of weather patterns.
- The M3 trap collected more particulate matter that was probably derived from zooplankton, as compared to the M2 trap, which contained more material derived from phytoplankton.
- Cholesterol is the major neutral lipid deposited to sediment traps at both M2 and M3. The major source of cholesterol is probably zooplankton fecal pellets.
- Weather affects the timing of blooms, at the ice edge versus open water, and the onset of fall mixing. During cold years, phytoplankton bloom in late April-early May at the receding ice edge. The input of diatoms and their fatty acids to sediment traps is greater in cold years. During warm years, an open water bloom occurs in late May or early June. The input zooplankton fatty acids and neutral lipids dominates lipid fluxes during warm years.

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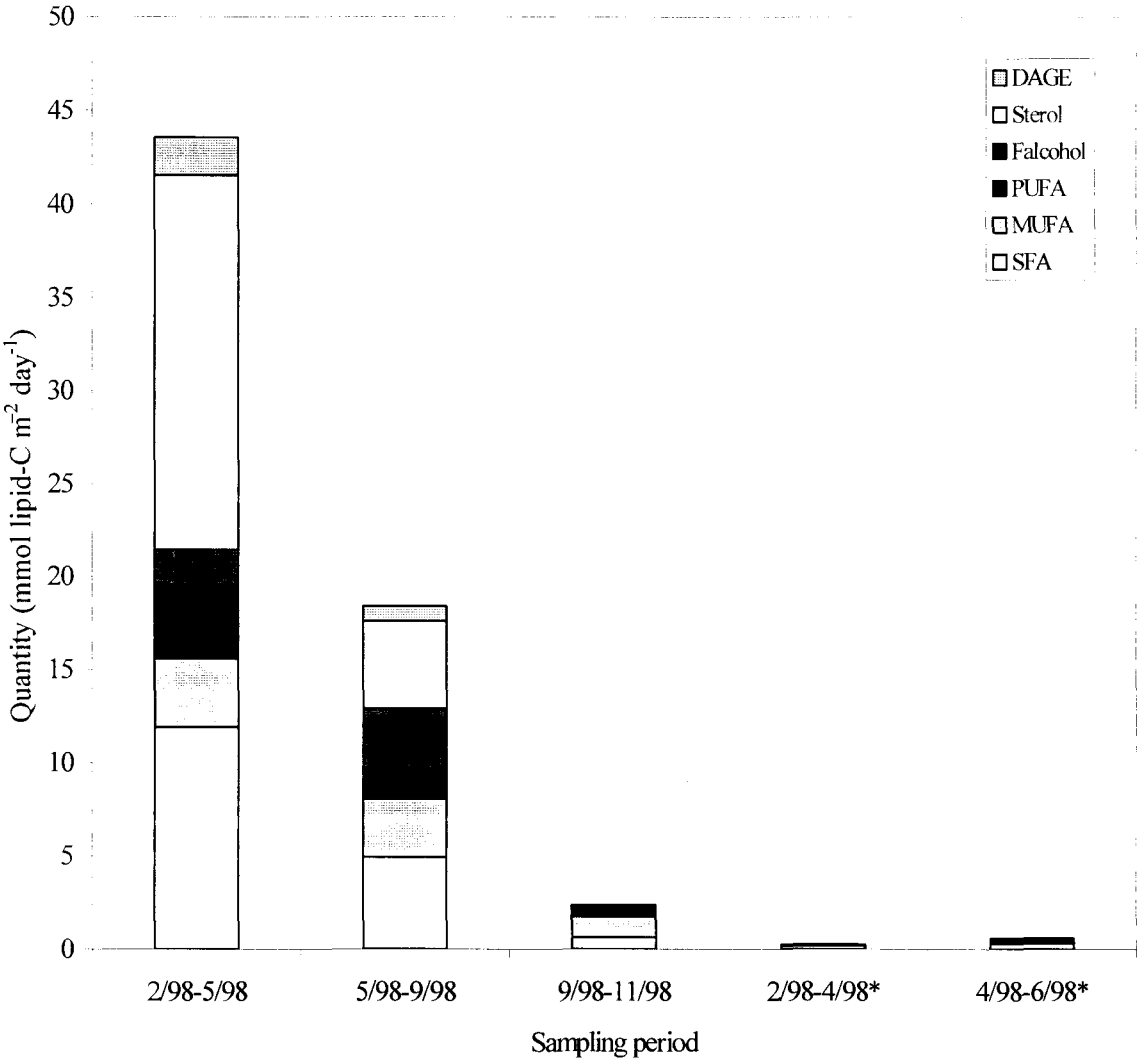


Figure 3.1a. The quantities of lipids collected at site M2 in 1998 and M3 in 1998. M3 samples are indicated with an asterisk (*). Lipids include the fatty acids SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), and neutral lipids, FALC (fatty alcohols), sterols and DAGE (diacylglyceryl ethers).

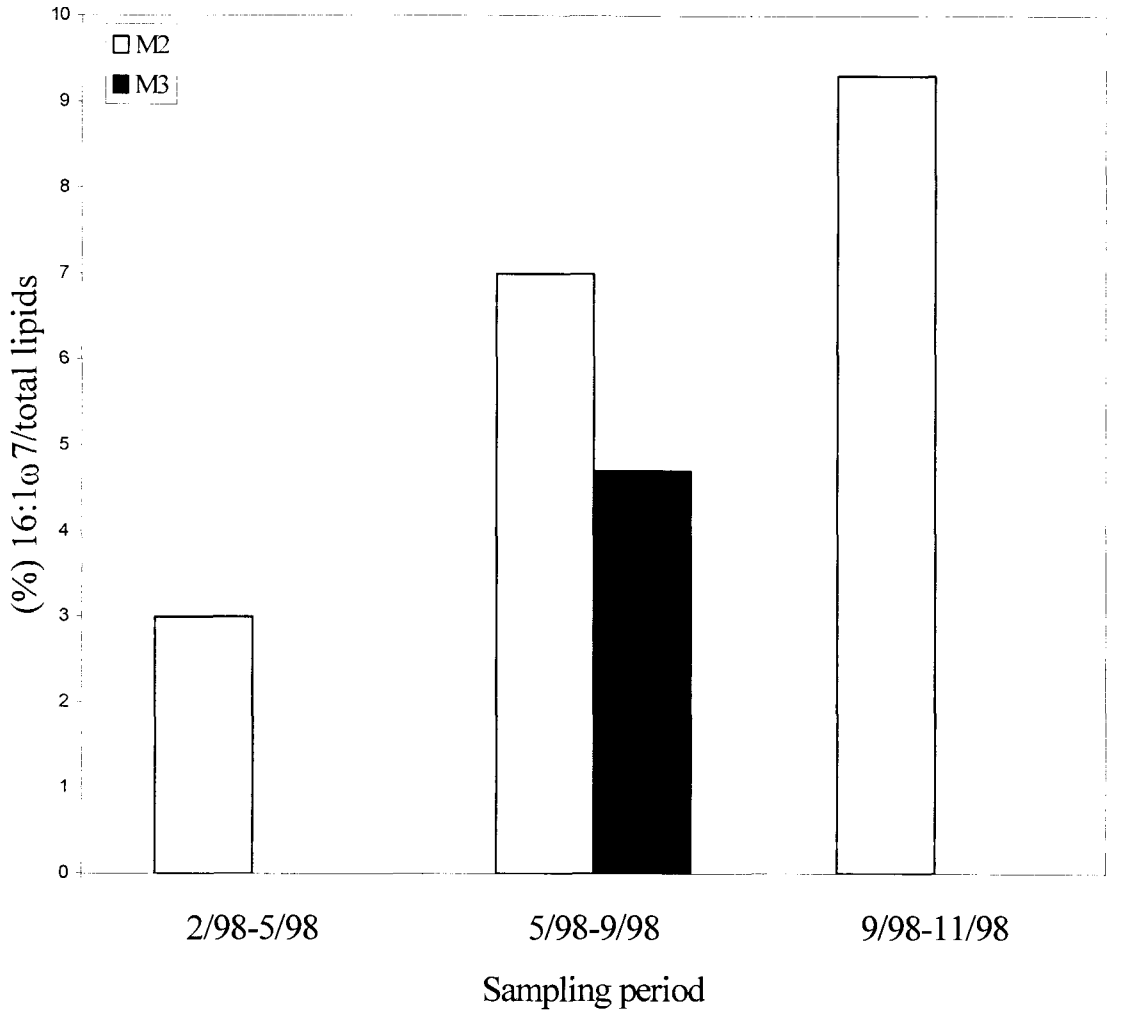


Figure 3.1b. Percent 16:ω7 fatty acid of total lipids for M2 in 1998 and M3 in 1998. This fatty acid is considered an indicator of diatoms.

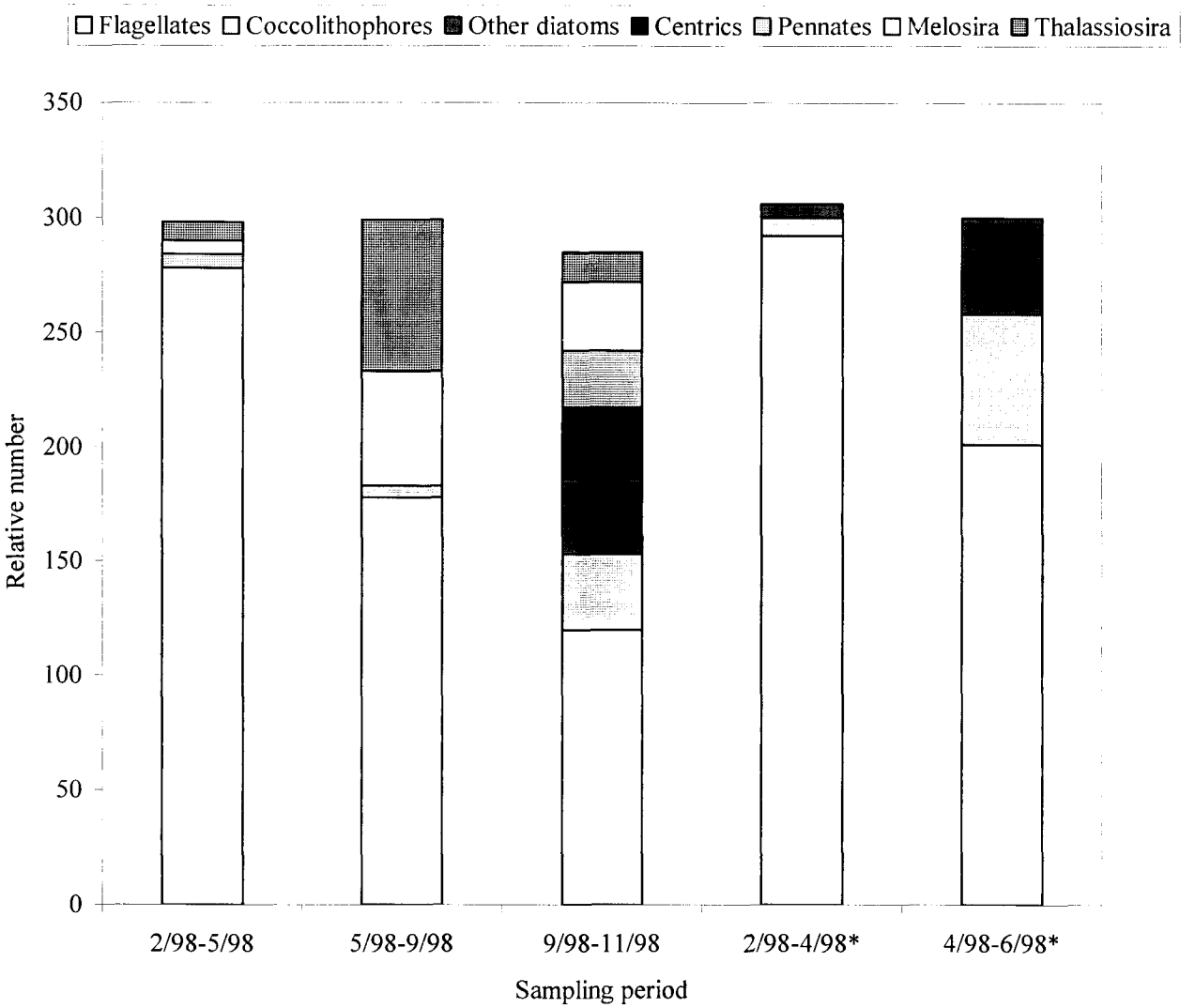


Figure 3.1c. The relative number of phytoplankton in each sampling period at M2 and M3 in 1998. M3 samples are indicated with an asterisk (*). Diatoms included under the heading "centrics" include all unidentified centric diatoms. Those termed "pennates" are the unidentified pennate diatoms. "Other diatoms" are identified diatoms such as *Coscinodiscus* spp., *Chaetoceros* spp., *Odontella* spp., *Rhizoselenia* spp., whose numbers were not great enough to justify a separate heading.

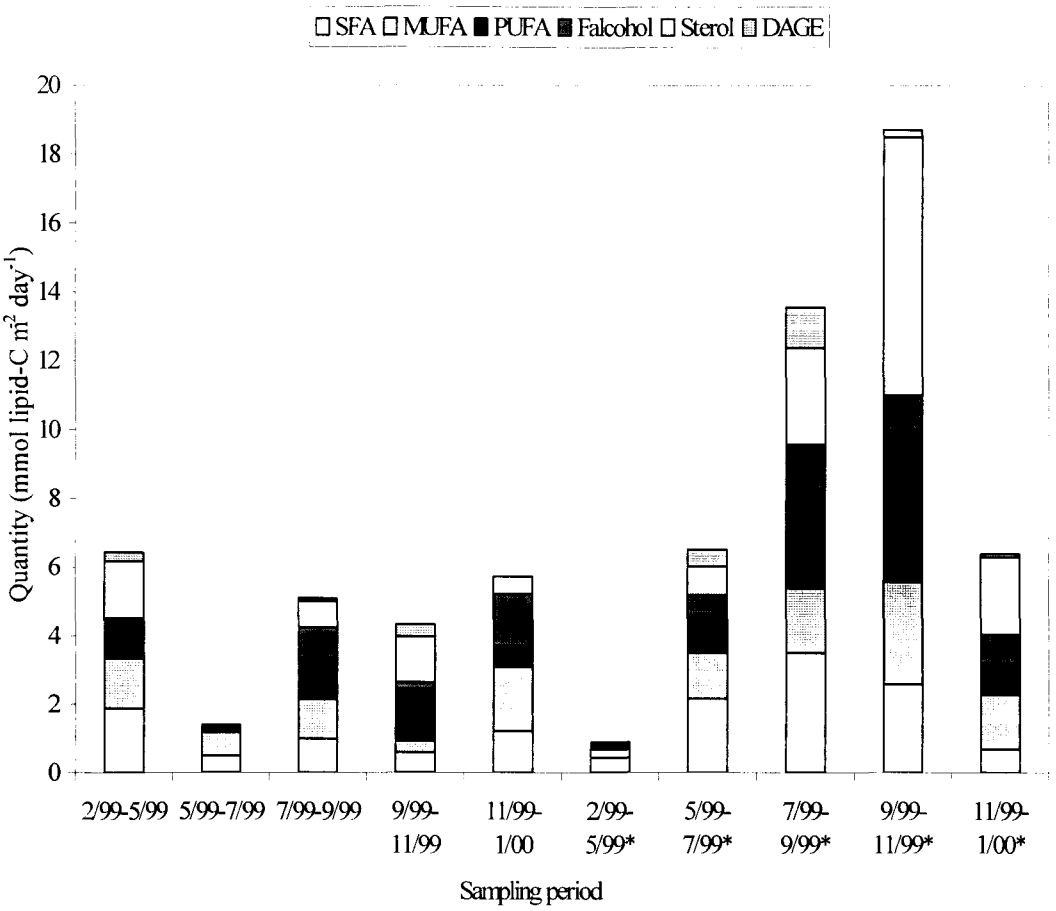


Figure 3.2a. The quantities of lipids collected at site M2 in 1999 and M3 in 1999. M3 samples are indicated with an asterisk (*). Lipids include the fatty acids SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), and neutral lipids, FALC (fatty alcohols), sterols and DAGE (diacylglyceryl ethers).

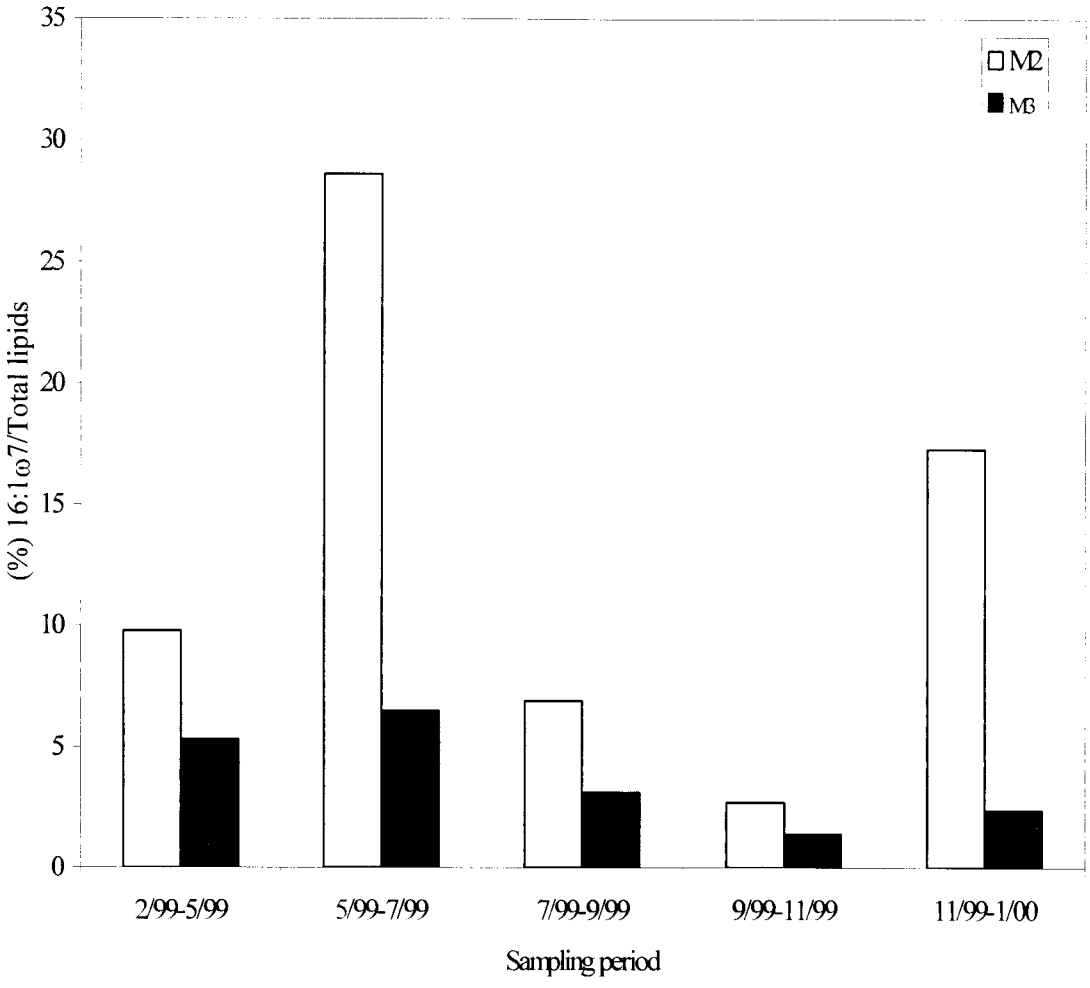


Figure 3.2b. Percent 16:ω7 fatty acid of total lipids for M2 in 1999 and M3 in 1999. This fatty acid is considered an indicator of diatoms.

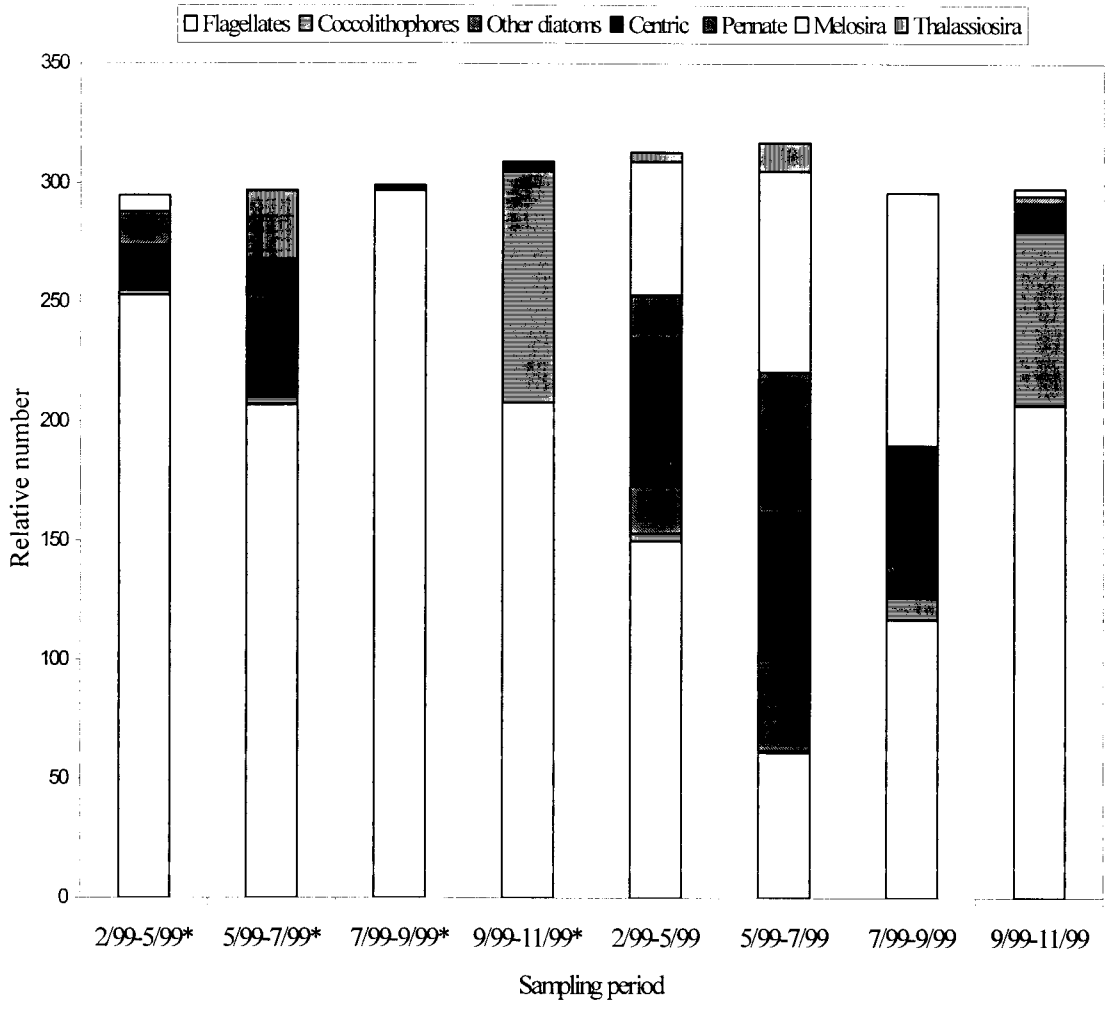


Figure 3.2c. Phytoplankton counts from M2 and M3 in 1999.

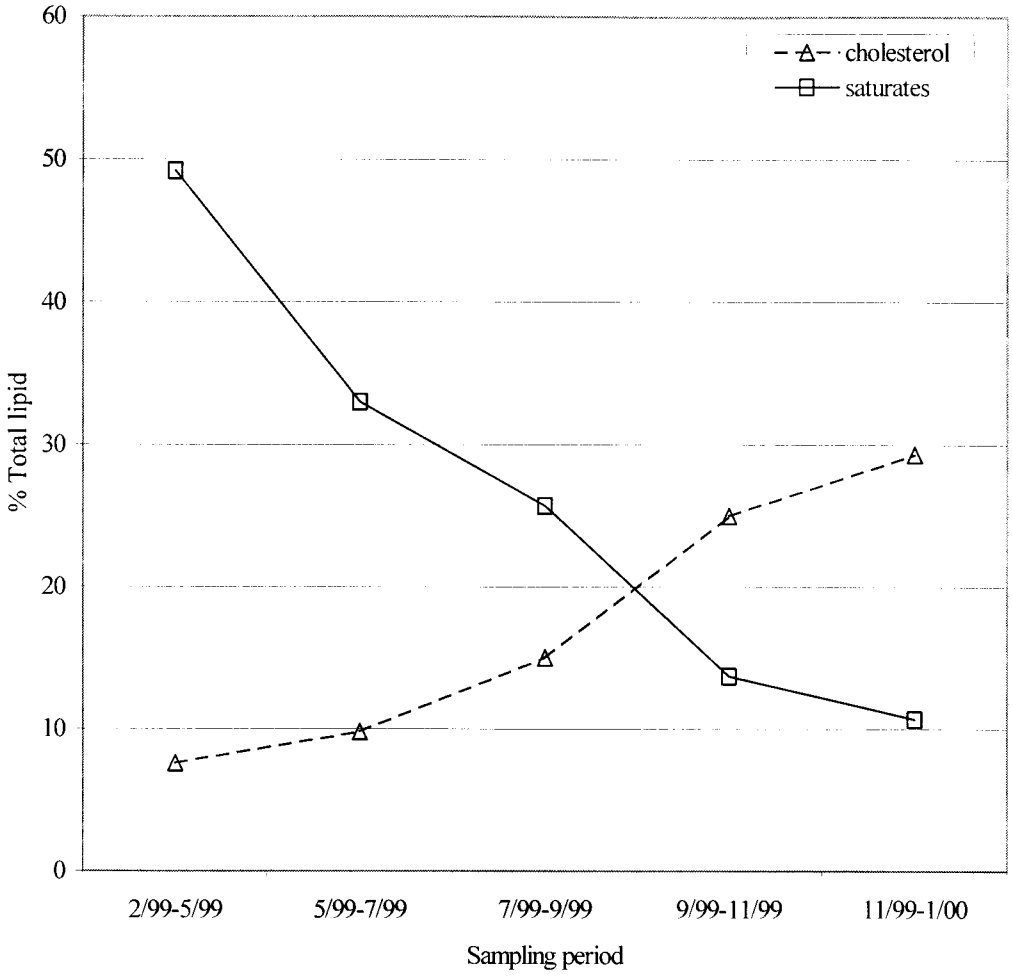


Figure 3.3. The percent cholesterol versus the percent saturated fatty acids from 2/99-1/00 at site M3. The lipids were negatively correlated.

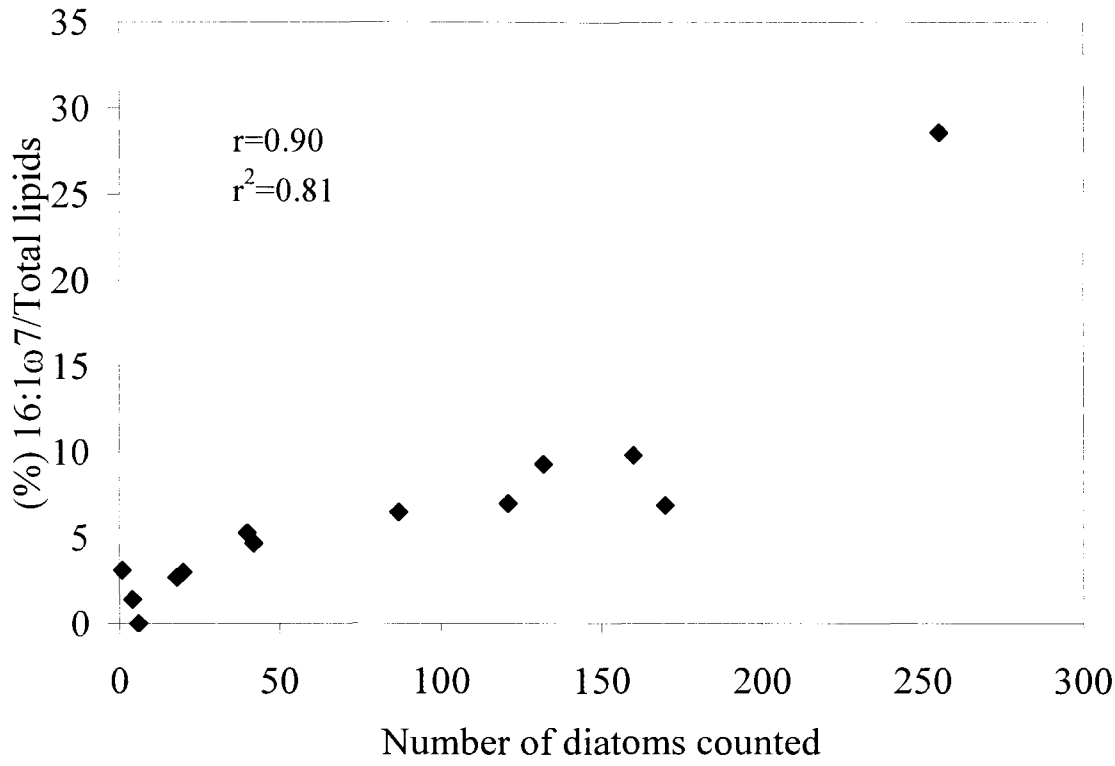
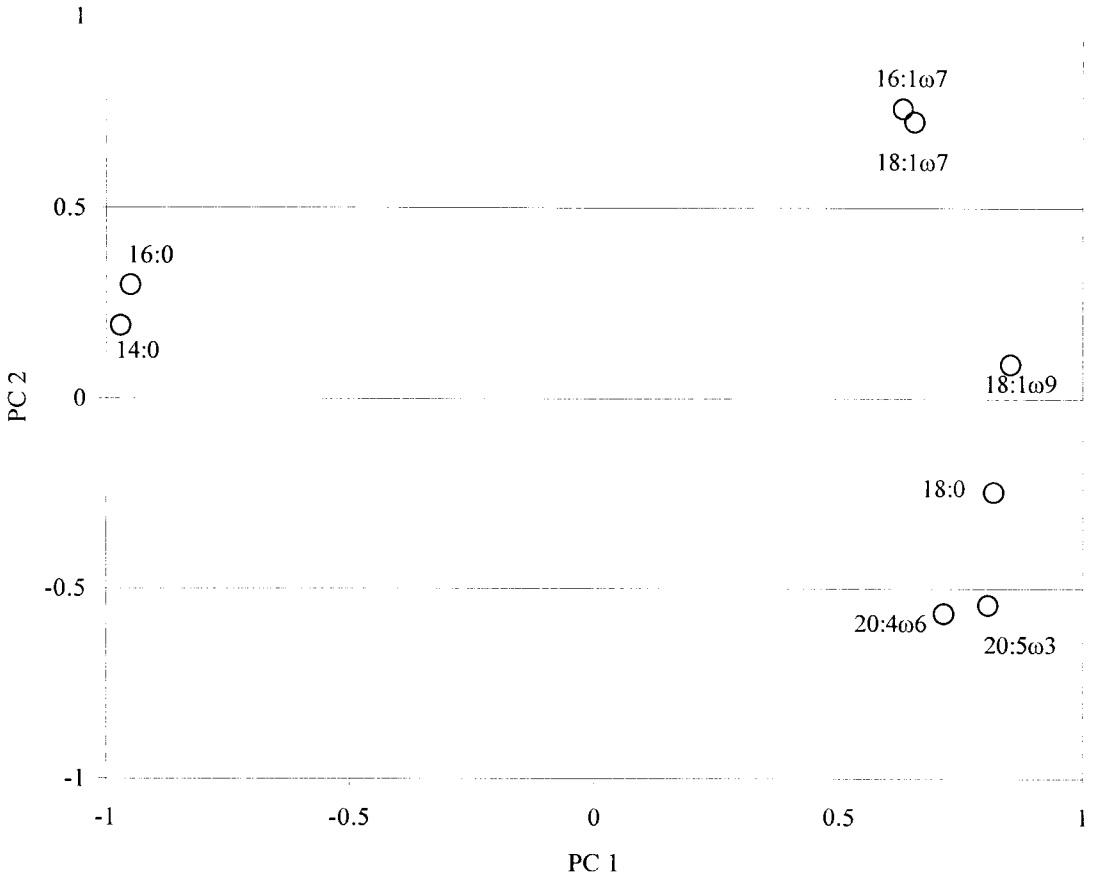


Figure 3.4. The number of diatoms counted in each sediment trap sample from sites M2 and M3 for 1998 and 1999 were plotted against the percent 16:1ω7 in the trap samples.



Figures 3.5a. Principal components analysis of lipid data from M3 sediment trap samples. Principal components analysis was used to show relationships between lipid groups in sediment trap samples. Variable loadings or coefficients from the two most influential principal components are plotted against each other.

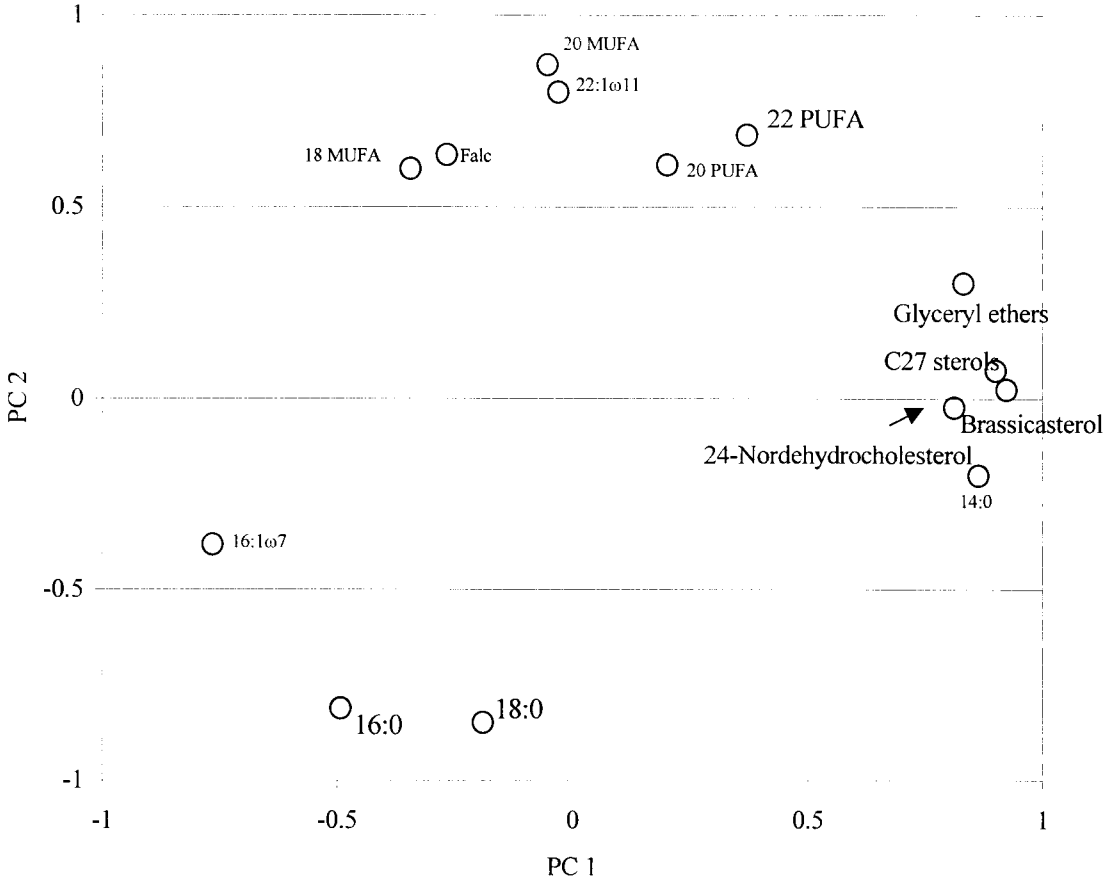


Figure 3.5b. Principal components analysis of lipid data from M2 1998 sediment trap samples.

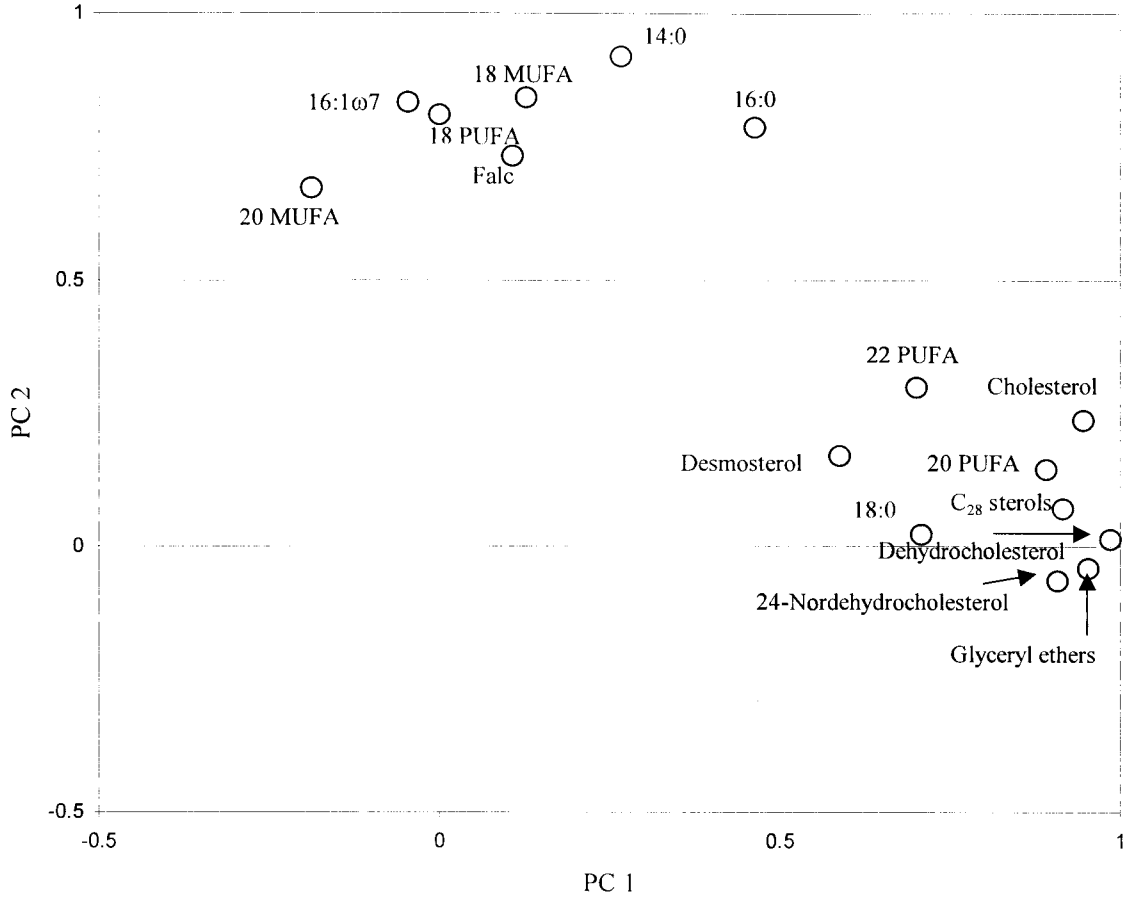


Figure 3.5c. Principal components analysis of lipid data from M3 1999 sediment trap samples.

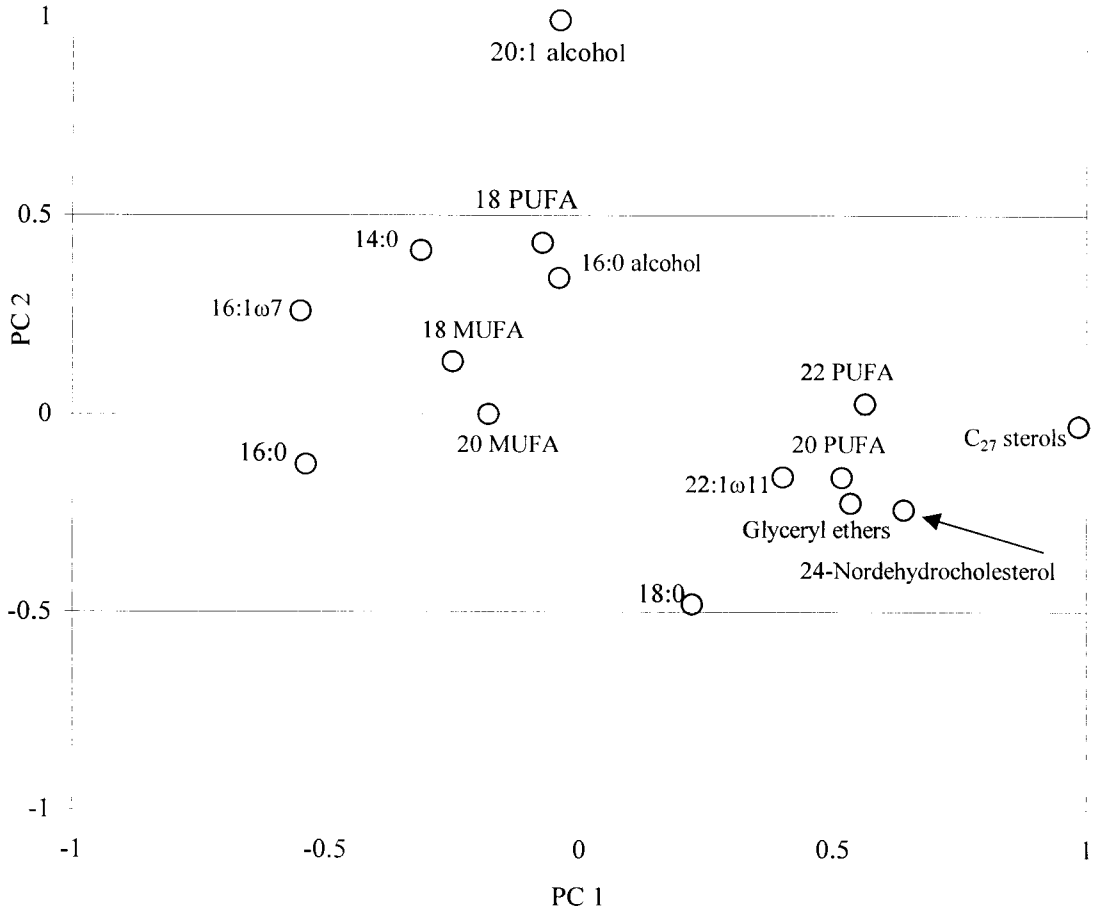


Figure 3.5d. Principal components analysis of lipid data from M2 1999 sediment trap samples.

Chapter 4.

Lipids as indicators of phytoplankton and zooplankton dynamics over the southeast Bering Sea shelf*

Key Words: Lipids, bacillariophyceae, biomarkers, calanoid copepods, fatty acids, fatty alcohols, sterols, sediment traps, phytoplankton, wax esters, zooplankton, Alaska, southeast Bering Sea

Abstract

The fatty acid and neutral lipid content of sinking organic matter was measured for samples collected over the southeast Bering Sea middle shelf using time-series sediment traps. The traps have been deployed year-round since April 1997. This paper focuses on lipids collected by the trap from April 1997 through January 2001. During two of the years, 1997 and 1999, sea ice retreated in late spring, resulting in ice edge blooms from late April to early May. Ice receded earlier in 1998 and 2000, so phytoplankton bloomed in open water in late May. These bloom dynamics affected the coupling of phytoplankton and zooplankton production, the type of organic matter sinking to the trap and its lipid content. Lipids indicating greater phytoplankton input were high during the ice-edge bloom years. In 1997, the most diverse population of diatoms was present of all the years. The highest percentage of the fatty acid 16:1ω7, associated with diatom input, was found, and ω3 polyunsaturated fatty acids (PUFA) and 16 carbon PUFA unique to diatoms were also present. The 1999 samples had the highest relative number of diatoms of any year, and high concentrations of 16:1ω7 and

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*Smith, S. L., and Henrichs, S. M. Lipids as indicators of phytoplankton and zooplankton dynamics over the southeast Bering Sea shelf. To be submitted to Cont. Shelf Res.

ω3 PUFA such as 20:5ω3 and 22:6ω3. Conversely, in 1998 and 2000, there was greater coupling between phytoplankton and zooplankton, and much of the material collected was fecal pellets. The major lipid collected in both years was cholesterol.

4.1. Introduction and Study Area

The southeast Bering Sea shelf is the broadest continental shelf outside the Arctic (Coachman, 1986). Over 500 km wide, it is a flat, featureless plain with mean bottom slopes of less than 0.2×10^{-3} (Askren, 1972). However, two zones of significantly steeper bottom slopes were identified as the cause of distinct physical regimes (Askren, 1972). These zones are centered at the 50 m and 100 m isobaths, and they help, together with density fronts, to divide the shelf into three domains of differing hydrography: the coastal domain, the middle shelf and the outer shelf. The middle domain, the site of the study, is separated from the coastal or "inner" domain by the inner front (Schumacher *et al.*, 1979). On the seaward side, the middle shelf is separated from the outer shelf by the middle front (Coachman and Charnell, 1979). These fronts are not fronts in the strictest sense, but are rather wide zones, much broader than the water is deep, in which the horizontal temperature and salinity gradients are strong relative to other areas (Coachman, 1986).

Tides in this region, which are the mixed, semidiurnal type, have a range of more than 1 m. Tidal currents drive the circulation of the middle domain. The water mass of the central shelf is distinguished by several characteristics (Kinder and Schumacher, 1981; Coachman, 1986), the most important among them being a two-layered vertical structure present during most of the production season. The top layer (10 m to 40 m) forms from melting ice and a decrease in winds during the winter, or from solar insolation during spring and summer. The bottom layer, segregated from other water masses by density fronts, is cold and homogenous. Other features of

central domain water include small horizontal temperature and salinity gradients within the domain, and injections of freshwater supplied mainly by ice melt and rain.

The ecosystem is highly productive. Productivity in this marginal sea is subject to seasonal and interannual variability, mostly due to changes in weather patterns (Hunt *et al.,* 2002). It can be marked by extreme changes in environmental conditions, from low light levels and total ice coverage in some winters, to the high light levels and warm sea surface temperatures of summer. The seasonality of this ecosystem, located at 56°53'N, 164°02'W, affects the ecology of phytoplankton, zooplankton and their predators in addition to the trophic interactions between predators and their prey. For example, the mid- to late-May spawning of *Thysanoessa raschi*, a euphausiid with a large biomass on the middle shelf, follows the seasonal increase in water temperature (Smith, 1991). However, during years when the ice extent is greatest, the spring phytoplankton bloom begins in April, while the water temperature is still near 0° C. This results in a decoupling of the primary and secondary production.

Phytoplankton on the middle shelf include diatoms, such as *Coscinodiscus* spp., *Thalassiosira* spp. and *Melosira sulcata* (Allen, 1927; Kawarada and Ohwada, 1957; Alexander and Cooney, 1979), the coccolithophorid *Emiliania huxleyi* and various dinoflagellates. Smaller calanoid copepods, eg., *Pseudocalanus* spp. and *Calanus marshallae,* and the euphausiid *T. raschi* (which can comprise 88% of the zooplankton biomass in April at some locations; Smith, 1991) are typical consumers over the middle shelf.

This study was conducted at a site on the middle shelf, M2, where a sediment

trap has been moored year-round since 1997. Since 1995, M2 has been the focus of constant monitoring by NOAA's Southeast Bering Sea Carrying Capacity (SEBSCC) program, of which this research was a part. Past investigations of this area have included Outer Continental Shelf Environmental Assessment Program (OCSEAP; 1970s and 1980s) and Processes and Resources of the Bering Sea (PROBES; 1978-1981). These studies aided in clarifying weather patterns, the physical oceanography of the area and how they combine to drive this marginal ice zone's ecology and biology. More recently, changes in the Bering Sea climate, correlated with the Pacific Decadal Oscillation (PDO) and a regional "regime shift" (Hare *et al.*, 1997; Niebauer, 1998), have been identified.

Recently, SEBSCC scientists have proposed a new hypothesis, called the Oscillating Control Hypothesis (OCH), which states that Bering Sea ecosystem dynamics are driven by alternating warm and cold regimes (Hunt *et al.*, 2002). The theory is that during warm years (late May bloom), the phytoplankton-zooplankton blooms are coupled and zooplankton growth rates increase, so large piscivorous fish such as walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) have abundant food and thrive – a top-down control of zooplankton and forage fish. During cold years (March or April bloom), the decoupling of the phytoplankton and zooplankton blooms limits secondary production and piscivorous fish production, a bottom-up control.

This study uses lipids collected in sediment traps as indicators of phytoplankton and zooplankton bloom events, bacterial decomposition, and processes involved in the

sinking of organic matter to depth. The aim is to show how weather affects both phytoplankton and zooplankton lipids in sinking particles; these patterns are interpreted as indicators of zooplankton consumption of the phytoplankton production and other facets of the Bering Sea's food webs.

4.2. Lipid Biochemistry

Lipids are synthesized by all organisms. Because the lipid biochemistry of plant and animals exhibits considerable diversity, lipids are useful as tracers in food webs, in tracking carbon flow from primary producers to herbivores and up to higher trophic levels. In the marine system, phytoplankton, bacteria and animals synthesize lipids, primarily fatty acids. All of these organisms can produce saturated fatty acids. They can also synthesize monounsaturated fatty acids aerobically, via the Δ^9 desaturase enzyme. This enzyme acts specifically on 16:0 and 18:0 and creates a double bond between the ninth and tenth carbons from the carboxyl end. The products of this pathway are 16:ω7 (palmitoleic acid) and 18:1ω9 (oleic acid). Although prokaryotes, photosynthetic eukaryotes and non-photosynthetic eukaryotes may synthesize unique fatty acids, they are limited in the kinds of fatty acids they can produce. For instance, although the chain length of most fatty acids is even-numbered, bacteria produce odd-chained fatty acids such as 15:0 and 17:0. Marine phytoplankton possess enzymes that enable them to continue desaturating monounsaturated fatty acids multiple times, thereby producing polyunsaturated fatty acids using the 9,12,15 pathway. Animals do not have this capability (Conte, 1990).

In marine environments, primary producers such as Bacillariophyceae (diatoms) may synthesize fatty acids unique to them, such as 16:4ω1 and other 16 PUFA, C_{25} and C_{30} highly branched isoprenoid (HBI) alkenes and 5α stanols (Volkman *et al.*, 1998). Diatoms are also high in less unique compounds like 16:0, 16:1ω7 and 20:5ω3 fatty acids. Dinophyceae (dinoflagellates) and Haptophyceae (mainly *Phaeocystis pouchetti* and *Emiliania huxleyi* in the Bering Sea) possess 18:5ω3 fatty acids and 5α-stanols. Coccolithophorids such as *Emiliania* also have distinctive C_{37}-C_{39} alkenones and alkenes among their suite of unusual lipids (Volkman *et al.*, 1980, 1998; Conte *et al.*, 1994). Herbivorous calanoid copepods often contain wax esters as 80% of their total lipids. They synthesize these *de novo*, as metabolic reserves and for buoyancy, but predominantly for egg production, especially in environments where food supply is highly seasonal. Euphausiids have both wax esters and triacylglycerols.

Sterols also can be species specific. C_{29} and C_{30} sterols such as dinosterol dominate dinoflagellate sterol profiles. Diatoms are high in 24-methylcholesta-5,22(E)-dien-3β-ol, which was once termed diatomsterol. Some may contain 24-methylcholest-5-en-3β-ol and 24-methylcholesta-5,24(28)(E)-dien-3β-ol. Consumers in marine ecosystems usually have cholesterol as their major sterol.

Terminology used to describe fatty acids gives the number of carbon atoms, the number of double bonds and the position of double bonds from either end. The Δ notation refers to the molecule and double bond positions beginning from the carboxyl

end, whereas the ω notation counts the double bond position starting from the terminal methyl group. For instance, α-linolenic acid, a fatty acid possessing 18 carbon atoms and 3 double bonds would be written $18{:}3\Delta^{9,12,15}$ in Δ notation. The double bonds are between carbons 9 and 10, carbons 12 and 13, and carbons 15 and 16, counting from the carboxyl end. Using ω notation, 18:3ω3 translates to 18 carbons atoms and 3 double bonds, with the first double bond position originating 3 carbons from the terminal methyl group. Double bonds are methylene interrupted. In this chapter, ω notation will be used when referring to fatty acids.

4.3. Methods

4.3.1. IRSC Sediment Traps

Two indented rotating sphere sediment traps, equipped with an eleven-sample carousel, collected a time series of sinking particles (Peterson *et al.*, 1993). A feature of this trap design is the indented rotating sphere, the function of which is to exclude swimmers from sample tubes. Most samples were swimmer-free. On occasion, one or two small copepods were found and picked out of samples. In a single event, a large number of pteropods were deposited into a September 1999 collection tube at M2. The traps were moored at two sites over the Bering Sea shelf. Trap M2 was deployed at middle shelf site M2 (56°53'N, 164°02'W; Figure 1.1), and was moored at 35 meters where the water depth was 70 m. The trap was recovered and redeployed twice a year, during a winter cruise in February and during a fall cruise in September. Prior to trap

deployments 5 g NaCl and 50 mg $HgCl_2$ were placed in sample tubes to retard bacterial activity. Sample collection intervals were two or three weeks, depending upon projected influx of material to the traps. Upon retrieval, samples were stored in pre-combusted sample bottles and frozen until split for analysis.

4.3.2. Microscopy

A Zeiss Telaval 31 inverted microscope was used to count and identify phytoplankton in trap samples. Ten ml of each subsample was preserved in 5% formalin. An aliquot of each subsample was thoroughly mixed, dispersed in a 25-ml capacity plate-settling chamber, and allowed to settle for 24 hours. Phytoplankton that settled on the plate were counted up to a total of 300. Whenever possible, phytoplankton were identified to species. Other particulate material, such as skeletal remains, tests and fecal pellets (zooplankton and dinoflagellate), were noted.

4.3.3. Lipids

Lipids were extracted from sediment trap samples using Soxhlet extractors and a solvent system of 2:1 CH_2Cl_2:MeOH (Wakeham *et al.*, 1997). Sediments were extracted for 24 hours. Following extraction, a 5% NaCl solution was added. The solvents and water were transferred to separatory funnels, and the lower, organic phase was separated and filtered using a 0.45μm Teflon® filter. The filtered solvent was placed in a 50 ml Teflon® test tube and evaporated to dryness using a centrifugal evaporator. Two ml of hexane:$CHCl_3$ were added and the sample was frozen and stored at -40° C until saponification.

A 100-µl or 200-µl aliquot (depending upon the amount of material) of each total solvent extract was transferred to a pre-combusted test tube. The total solvent extract (TSE) was evaporated to dryness then saponified using 2 ml 5% w/v KOH in 80/20 methanol/glass distilled water and heated at 60° C for 3 hours. One ml of water was added, and then the neutral lipid fraction was extracted from the TSE using 4:1 hexane:$CHCl_3$. Recovered neutrals were derivatized using 50 µl BSTFA to form TMS esters. Samples were made up to a volume of 2 ml. Cholestane was used as an internal GC standard. The TSE was acidified with 0.3 ml HCl and fatty acids were extracted with 4:1 hexane:chloroform. Acid fractions were methylated with 3 ml 10:1:1 CH_3OH:HCl:$CHCl_3$ and heated for 1 hour at 100° C. One ml glass distilled water was added, and the fatty acid methyl esters were extracted 3 times with the hexane solvent. Solutions were then made up to 2 ml volume, and methylnonadecanoate was added as an internal standard.

4.3.4. Gas chromatography

TMS derivatives and fatty acid methyl esters were analyzed by GC-MS (gas chromatography-mass spectrometry) using a Hewlett-Packard 5890 Series II Plus Gas Chromatograph. A 30 m X 0.25mm inner diameter capillary column with splitless injection was used with helium as the carrier gas. Injection temperature was 250° C and detector transfer line temperature was 300° C for fatty acids. Initial oven temperature was held at 180° C for two minutes then ramped at a rate of 2° C/min to 250° C. The flow rate was 1 ml/min. For sterol and fatty alcohol derivatives, injection

temperature was 250° C and detector temperature was 290° C. Initial oven temperature was held at 180° C for four minutes, and then ramped at 3° C/min to 310° C.

4.4. Results

Sediment trap samples from 1997 and 1999 had several similarities. In particular, the quantity of total lipids collected was similar at around 20 mmol lipid-C m^{-2} day^{-1}. However, in comparing the two years, note that data from 2/10/99 are available, but the collection period in 1997 lasted from 4/22/97-1/98. Only lipid data from 4/22-10/27 are reported. The sample from 10/27-11/10 (sample 15) was inadvertently pyrolyzed during Soxhlet lipid extraction. Samples 16 and 17 (collection dates 11/10-12/8 and 12/8-12/22) contained lipids, but the amount could not be calculated because the quantity of carbon was not determined. It is estimated that the quantity of lipids in samples 15 and 16 were half the quantity of sample 14. Sample 17 contained less than one-fourth the quantity of sample 14.. Samples 18-22, from 12/22 through 1/98, contained only trace amounts of organic carbon.

The concentration of the diatom indicator 16:1ω7 was twice as high in 1997 (14.2%) and 1999 (13.1%) as it was in either 1998 (6.5%) or 2000 (7.0%) (Figure 4.1a). The same was true of omega-3 polyunsaturated fatty acids in the sediment trap material (Figure 4.1b). PUFA, especially 20:5ω3, were more than twice as abundant during the ice edge years, 1997 (17%) and 1999 (21%), than the open water bloom years, 1998 and 2000 (both 9%). In addition to this, monounsaturated fatty acids and polyunsaturates were negatively correlated in both 1997 ($r=-0.84$; $r^2=0.71$) and 1999

(r=-0.81; r^2=0.66), but were highly positively correlated in 1998 (r=0.995; r^2=0.990) and 2000 (r=0.996; r^2=0.993) (Figures 4.2a-4.2d). The percent of cholesterol in 1998 and 2000 was greater than in either 1997 or 1999. Fatty alcohols, usually indicators of zooplankton wax esters, increased in concentration over the four-year period (Figures 4.3a, 4.3b).

When the different lipid classes from 1997 and 1999 are combined (Figures 4.4a,4.4c), the dominant lipid class is saturated fatty acids, which were collected at a rate of about 2.5 mmol lipid-C m^{-2} day^{-1}, greater than the rate for monounsaturates, polyunsaturates and sterols. Fatty alcohols and diacylglyceryl ethers are smaller percentages of the total lipids. Fatty acids were more concentrated in 1997 and 1999 sediment trap samples than neutral lipids. Saturated fatty acids were dominant in 1998 samples, but the quantity of sterols collected exceeded all other lipid classes. PUFA levels were less than 10% of the total lipids. In samples from 2000, sterols were the dominant lipid class, followed closely by fatty alcohols and MUFA (monounsaturated fatty acids), while PUFA quantities were relatively low. Cholesterol was the dominant lipid at the beginning of 1998 and in 2000 percentage-wise, but its concentration diminished over the year. For two of the years, 1997 and 2000, the quantity of lipids collected by the trap was greatest in the fall. This was not the case for the 1999 and 1998 trap samples. For these two years, late winter-early spring marked the highest lipid collection.

Figure 4.5 shows the total quantity of lipids collected in the trap each year. The quantity collected in 1998 (64.3 mmol lipid-C m^{-2} day^{-1}; 2/98-11/98) was nearly double

that collected in 2000 (36.4 mmol lipid-C m^{-2} day^{-1}; 4/00-2/01) and triple the amounts of 1997 (20.8 mmol lipid-C m^{-2} day^{-1}; 4/97-10/97) and 1999 (22.9 mmol lipid-C m^{-2} day^{-1}; 2/99-1/00).

As shown in Figure 4.6, the number of diatoms counted in sediment trap samples was much greater in 1999 than in either 1997 or 1998. The 9/98-11/98 samples were the only ones from the previous two years to contain diatoms as more than 50% of the phytoplankton counted. In 1997, the sampling period 9/97-11/97 contained coccolithophorids and the samples were chalky white. The coccolithophorids were identified as *E. huxleyi* using scanning electron microscopy. However, as many of the coccolithophorids were broken up into 2 μm coccoliths, they were not counted during light microscopy.

4.5. Discussion

As stated earlier, two types of spring blooms occur over the southeast Bering Sea middle shelf: the first type is an ice edge bloom and the second is an open water bloom (Niebauer *et al.*, 1995). Ice edge blooms are triggered by water column stability at the receding ice edge, where less dense, fresher water sits atop more saline water, usually in April. Open water blooms occur when winds decrease and solar insolation increases warming surface waters and creating thermal stability, usually in May. Two of the four years studied, 1997 and 1999, experienced ice edge blooms and two years, 1998 and 2000, experienced open water blooms.

The monounsaturated fatty acid (palmitoleic acid) 16:1ω7 has long been

considered an indicator of Bacillariophyceae or diatoms (Dunstan *et al.*, 1994; Volkman *et al.*, 1998). The highest concentrations of 16:1ω7 in the organic matter usually occurred when the greatest number of diatoms was counted in the corresponding sediment trap sample. The data show that a greater quantity of diatoms sinks with an ice edge bloom than an open water bloom. Several factors may be responsible for this, the first being the stability of the water column as the ice retreats. The strong pycnocline results in nutrient depletion of surface waters (Niebauer *et al.*, 1995). This nutrient depleted condition, in turn, can lead to the production of more PUFA by diatoms (Zhukhov and Aizdaicher, 2001). Hence, 16:1ω7 and 20:5ω3 are negatively correlated. The negative correlation between monounsaturated fatty acids and polyunsaturated fatty acids (mainly 16:1ω7 and 20:5ω3) during 1997 and 1999 has also been noted by Zhukhov and Aizdaicher (2001) as being a feature of diatom fatty acids. This stems from the need to produce neutral lipids during times of nutrient supply and to produce polar, cellular lipids during times of nutrient depletion and resting cell formation. Important organic compounds needed for cell survival under unfavorable conditions accumulate during the resting stage period (Lichtle and Dubacq, 1984; Kuwata *et al.*, 1993; Peters and Thomas, 1996).

A second reason that diatom indicators are dominant during ice edge bloom years is that, according to Vidal and Smith (1986), middle shelf zooplankton such as the euphausiid *T. raschii* spawn in mid- to late-May, following the seasonal increases in temperature (Smith, 1991). Reproduction of calanoid copepods that live on the middle shelf such as *C. marshallae* begins following the phytoplankton bloom (Vidal

and Smith, 1986). Therefore, if a large biomass of zooplankton is not present, grazing is limited (Cooney and Coyle, 1982) and intact phytoplankton reach the trap. Supporting this scenario is the fact that 16:1ω7 was 28.6% of the total lipid in 5/99-7/99, during the height of the diatom influx. No significant zooplankton-derived sterols such as cholesterol or fatty alcohols (20:1 and 22:1) were present during this sampling period. Coyle and Pinchuk (2002a) found a lower abundance of *Calanus* during an 1999 inner front survey. The 1997 data reflected this same pattern. During the maximum collection of palmitoleic acid (33.7%), 4/22-5/6, cholesterol and fatty alcohols were a small percentage of the lipids, 6% and 2%, respectively.

The lower relative concentration of 16:1ω7 in 1998 and 2000 samples also could be due to several factors. First, it is possible that primary productivity over the Bering Sea middle shelf is less during an open water bloom, due to a less stable water column. This is unlikely because the amount of material collected by the trap was actually greater during these years. The mostly likely explanation is that 1998 and 2000 open water phytoplankton blooms coincided with the euphausiid and copepod maxima, resulting in substantial zooplankton grazing of the blooms (Coyle and Pinchuck, 2002b). The deposition of fatty acids versus cholesterol during ice edge versus open water blooms is consistent with the Oscillating Control Hypothesis (OCH; Hunt *et al.,* 2002).

All plants, fungi and most yeasts have the ability to synthesize PUFA *de novo* (Nes and Nes, 1980). Higher animals do not have this capability, and must obtain PUFA from their diet. Much of the PUFA in 1997 and 1999 sediment trap samples

apparently came directly from ungrazed phytoplankton. During periods of high lipid collection by the sediment trap, the PUFA concentration was low. During a fall phytoplankton bloom, 9/98-11/98, 16:1ω7 was a higher percentage of total lipid, but the quantity of lipid collected was the lowest of that year. Warm temperatures and high zooplankton growth rates could have led to early zooplankton ontogenetic migration, accounting for the lack of grazing of the fall diatom bloom. The generally low concentration of ω3 PUFA in 1998 and 2000 sediment traps could be due to tighter phytoplankton-zooplankton coupling and, hence, zooplankton assimilation of the ω3 PUFA. Zooplankton collected near M2 were high in PUFA throughout 1998 (Chapter 5), even in February.

According to Sargent and Henderson (1986), calanoid copepods continue to synthesize fatty acids (mostly saturated and monoenoic) while simultaneously assimilating dietary fatty acids (mostly polyunsaturated). Copepods are able to do this because of the production of wax esters; they reduce their synthesized fatty acids into fatty alcohols, in the process consuming NADH and NADPH, which inhibit fatty acid synthesis. Euphausiids also contain wax esters, although not as much as calanoid copepods. Zooplankton from warm years like 1998 contain more PUFA (Chapter 5). Also, the wax esters that are accumulated by zooplankton are not mobilized to a great extent during winter. The low percentage of PUFA could be due to higher zooplankton assimilation rates, at least in 1998. The positive correlation between MUFA and PUFA in trap material during open water years could be explained if zooplankton simultaneously assimilated PUFA from their diet and biosynthesized MUFA such as

20:1 and 22:1 for further reduction to fatty alcohols.

T. raschii, probably owing to its high biomass over the middle shelf, had a high input of lipids into the sediment trap. Calanoid copepods contain a higher concentration of 20 and 22 MUFA and often represent the highest biomass of over the shelf (Coyle and Pinchuk, 2002b). So an alternative explanation for the MUFA and PUFA correlation is the feeding history of the euphausiids and calanoid copepods. For instance, diatoms over the middle shelf have both MUFA and PUFA as equal portions (about one-third each) of their total lipids (Chapter 5). The animals could be accumulating fatty acids and transforming them into storage lipids such as triacylglycerols and wax esters.

The percentage of cholesterol was higher in 1998 and 2000 than it was in either 1997 or 1999. Harvey *et al.* (1987) showed that in calanoid copepods fed dinoflagellates, the excretion of cholesterol and sterols in general increased with the food supply. The highest collection of cholesterol by the trap in 1998 was during the 2/98-5/98 sampling interval. This implies that zooplankton were feeding extensively, since the cholesterol deposition during this period was the greatest of all four years. However, it is not clear what they were eating, since no phytoplankton bloom was detected by the fluorometer moored at M2 (Stabeno and Hunt, 2002). Unidentified flagellates were extremely abundant during this time period, perhaps explaining why the cholesterol concentration was so high. In addition, the fatty alcohols 20:1ω9 and 22:1ω11 were detected among the neutral lipids of *T. raschi* collected in early 1998 (Chapter 5). This indicates that the euphausiids were feeding on copepods, most likely

excreting a portion of the ingested cholesterol. In 2000, the highest quantity of cholesterol collected was during the 8/00-10/00 sampling period. Other animal-derived lipids such as 20:1ω9 and 22:1ω11 fatty alcohols were highly concentrated during this time frame, implying increased zooplankton input, and probably phytoplankton input.

The percentage of fatty alcohols descending to the sediment traps increased over the four-year period. The increase in fatty alcohols was from 2.5% in 1997 to 17% in 2000. Since calanoid copepods are the sole biosynthesizers of this lipid class over the middle shelf, an increase in this lipid flux to the trap may indicate an increase in their population. The year 2000 was warm and zooplankton growth rates were relatively high. The percentage of fatty alcohols was lower, but not the quantity. The OCH suggests that in warm years food web dynamics are driven from the top down, and that predators, not phytoplankton production, control copepod populations. Major predators of copepods are other plankton such as *T. raschii*, chaetognaths, scyphomedusan jellyfish, and juvenile pollock. Most of these animals excrete the wax esters or catabolize them, either oxidizing the fatty alcohols to fatty acids or excreting them in fecal material. In a study by Sargent *et al.* (1979), feces of herring and rainbow trout fed *Calanus finmarchicus* contained wax esters as 32.8% and 52.4% (respectively) of their total lipid. So, an increase in the percentage of fatty alcohols in sediment trap samples over the four years may represent an increase in predation.

In Figures 4.4a-d, which show the quantity each lipid class collected over the four-year study, 1998 had the highest input from sterols and saturated fatty acids. Since cholesterol is not as easily broken down as MUFA or PUFA, it would have a

longer lifetime in the sediments. MUFA and PUFA have a faster turnover time. Sterols and longer-chain compounds such as alkenones were found to be the highest percentages of lipids in deep sediment traps and in sediments versus plankton and shallow sediment traps (Wakeham *et al.*, 1997). While it may be true that organisms excreted more cholesterol in 1998, in addition to this, the higher percentage of cholesterol in the trap may be due to its being more refractory than polyunsaturated fatty acids.

Figure 4.5 shows the total quantity of lipids collected in the trap each year, with the 1998 quantities more than double those of the other years. Total lipid collected during the "warm" year averaged 100 mmol lipid-C m^{-2} day^{-1}; the amount collected during the "cold" year averaged only 43 mmol lipid-C m^{-2} day^{-1}. Since the lipids during the warm year were mainly from zooplankton, a greater flux of cholesterol, saturated fatty acids and fatty alcohols were collected in the trap over the four-year period, as expected.

Annually during August through September there is mixing of the water column, nutrient input and a fall bloom, resulting in a large increase in the quantity of sedimenting material. In 1997, 1998 and 2000, the fall quantity collected was nearly 20 mmol lipid-C m^{-2} day^{-1}. This was not the case in 1999. A bloom of coccolithophores covered the entire shelf in the fall. If the 1997 paradigm of bloom, grazing and deposition of high quantities of saturated fatty acids and cholesterol had been followed, an enormous amount of lipid would have been deposited into the sediment trap. Possible explanations for the lack of lipid deposition include that the

fall of 1999 was warm and mild. High pressure dominated and there was not a lot of wind to mix the water column and induce sedimentation of the coccolithophorids. Coccoliths are small (2 μm) and sink rather slowly in the absence of aggregation or vertical mixing. Another possible explanation is that fewer copepods and euphausiids existed to graze the bloom or that they were grazing selectively on diatoms (Olson and Strom, 2002).

Phytoplankton assemblages differed among the years 1997-1999. (Phytoplankton were not counted in 2000 because of lack of equipment). Fewer diatoms and more flagellates were collected by the sediment trap in 1997, except during the sampling period 4/22-5/6. The dominant species in trap samples shifted from *Thalassiosira* spp. in 1997 to *Melosira sulcata* in 1999. One possibility could be that nutrients were depleted earlier in 1997, quickly decreasing the primary production and supply to the trap. After the 1999 ice edge bloom began, nutrients did not decrease until later in the season (Whitledge and Rho). Another reason could be that zooplankton grazing pressure was higher in 1997. The 1997 phytoplankton consisted of a greater variety of diatoms, silicoflagellates and dinoflagellates, while those of 1998 and 1999 consisted of fewer diatom species and other phytoplankton in general.

4.6. Conclusions

Lipids found in the sediment trap support aspects of the Hunt *et al.* (2002) Oscillating Control Hypothesis. Fatty acids indicative of primary producers were found during ice-edge blooms. Lipids found during open water bloom years support

evidence for more zooplankton input, like increased cholesterol concentration and increased quantity collected in the traps.

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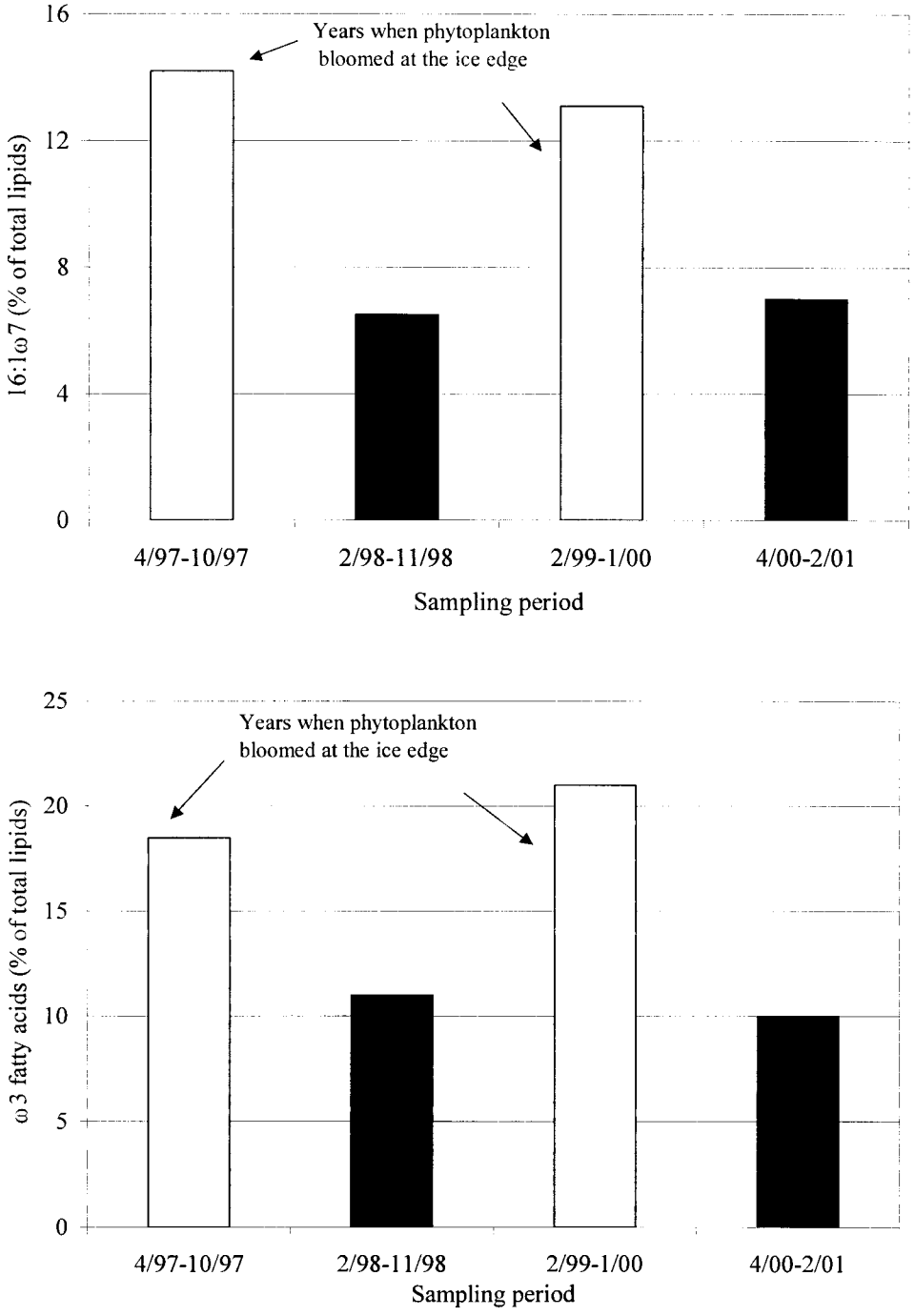
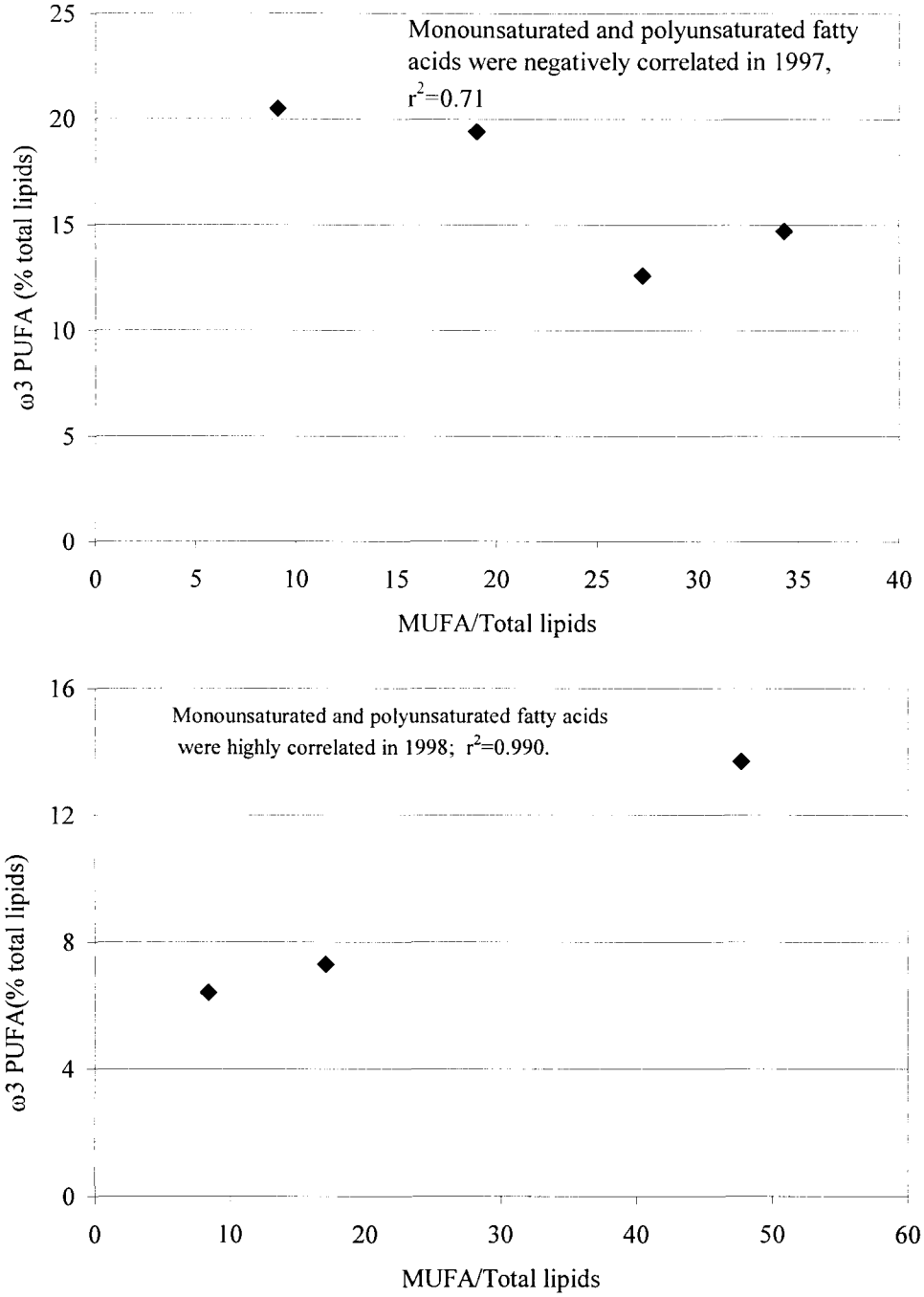
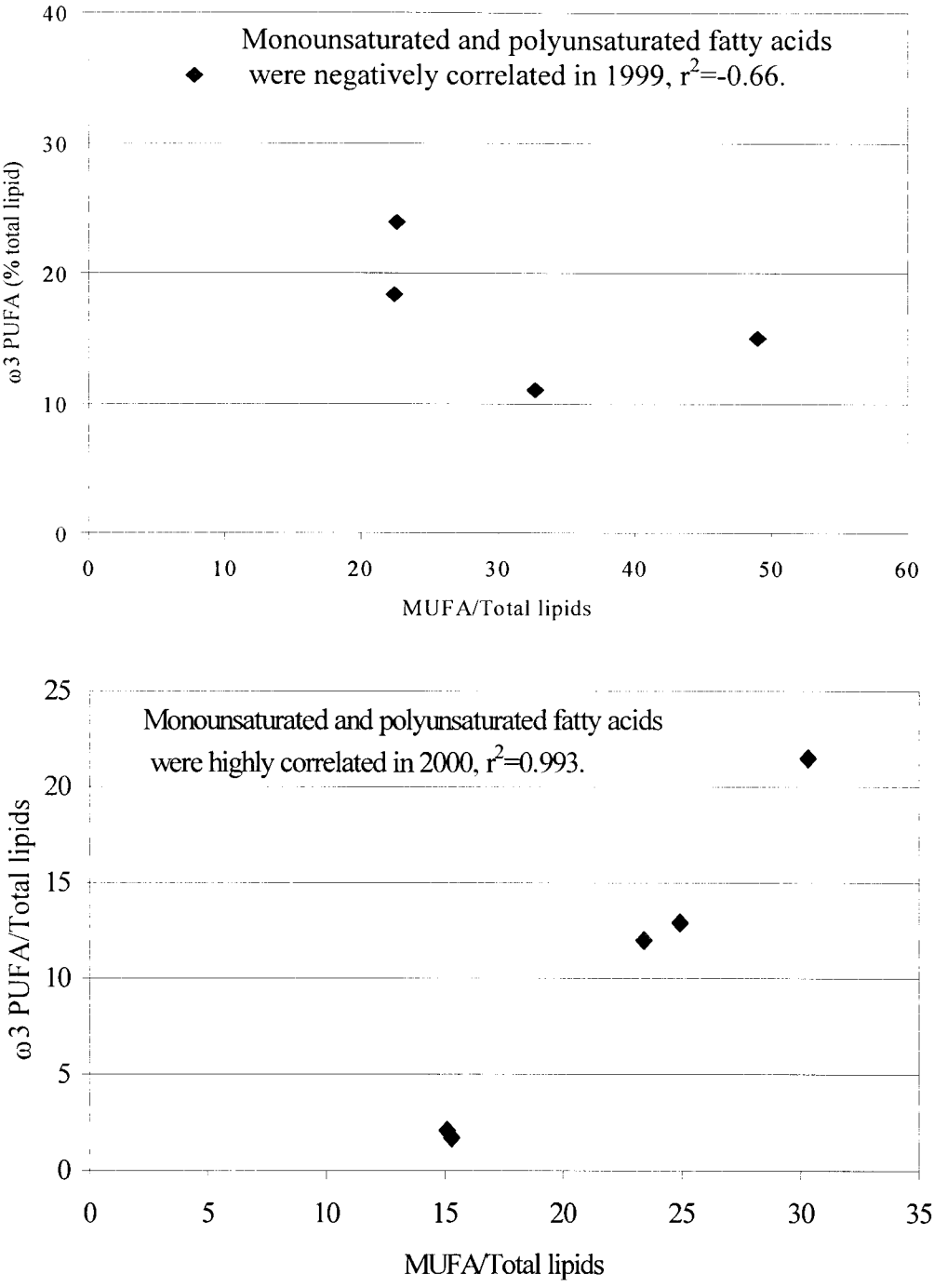


Figures 4.1a-b. Percent fatty acids in sediment trap samples 1997-2000, 16:1w7 (top) and ω3 (bottom). Cold years have higher 16:1ω7 and ω3 fatty acids.



Figures 4.2a-b. The correlation of the diatom fatty acid 16:1ω7 and polyunsaturated fatty acids in 1997-1998. The two were negatively correlated in 1997 and 1999 but positively correlated in 1998 and 2000. PUFA= polyunsaturated fatty acids. MUFA=monounsaturated fatty acids.



Figures 4.2c-d. The correlation of the diatom fatty acid 16:1ω7 and polyunsaturated fatty acids in 1999-2000. The two were negatively correlated in 1997 and 1999 but positively correlated in 1998 and 2000. PUFA= polyunsaturated fatty acids. MUFA=monounsaturated fatty acids.

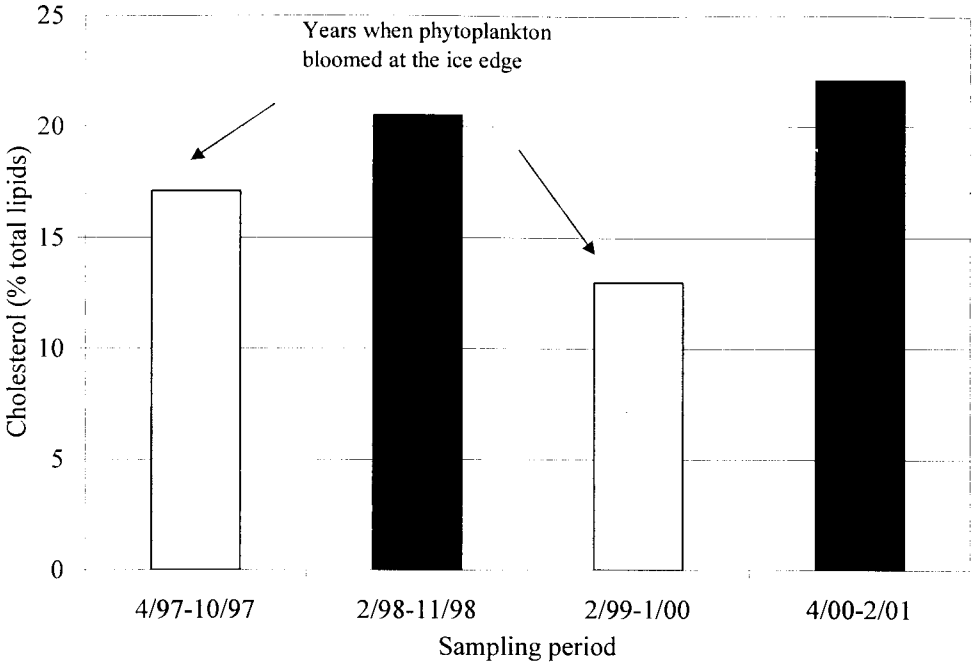


Figure 4.3a. The percentage of cholesterol in the total lipids. The percentage was greater in the open water bloom years 1998 and 2000 than in 1997 and 1999.

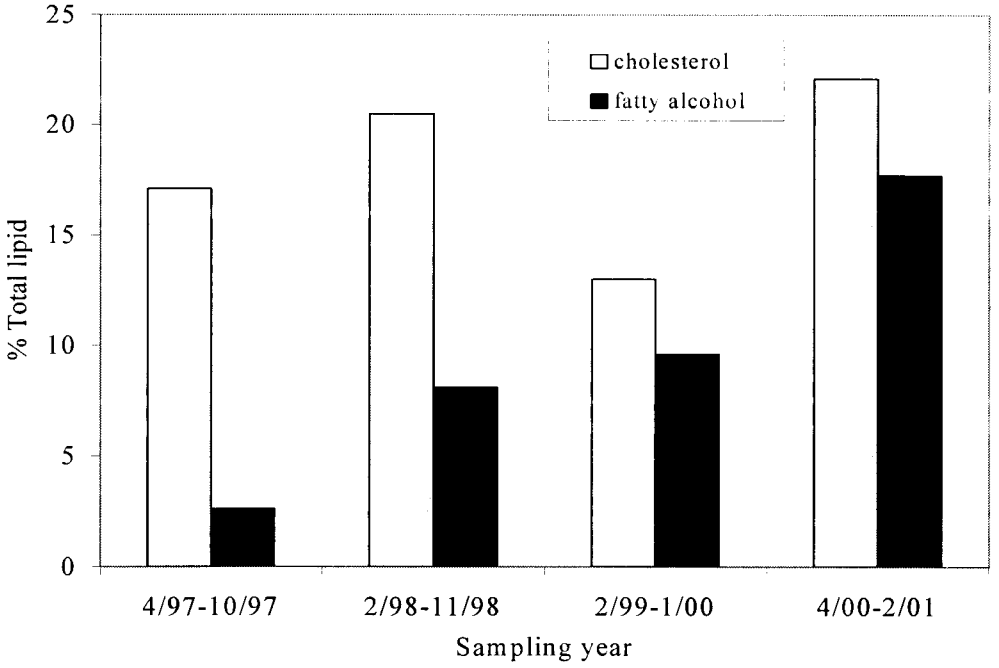


Figure 4.3b. The percentage of fatty alcohols in the total lipids. The percentage increased over the four-year period, from 2.5% of the total in 1997 to over 17% of total lipids in 2000.

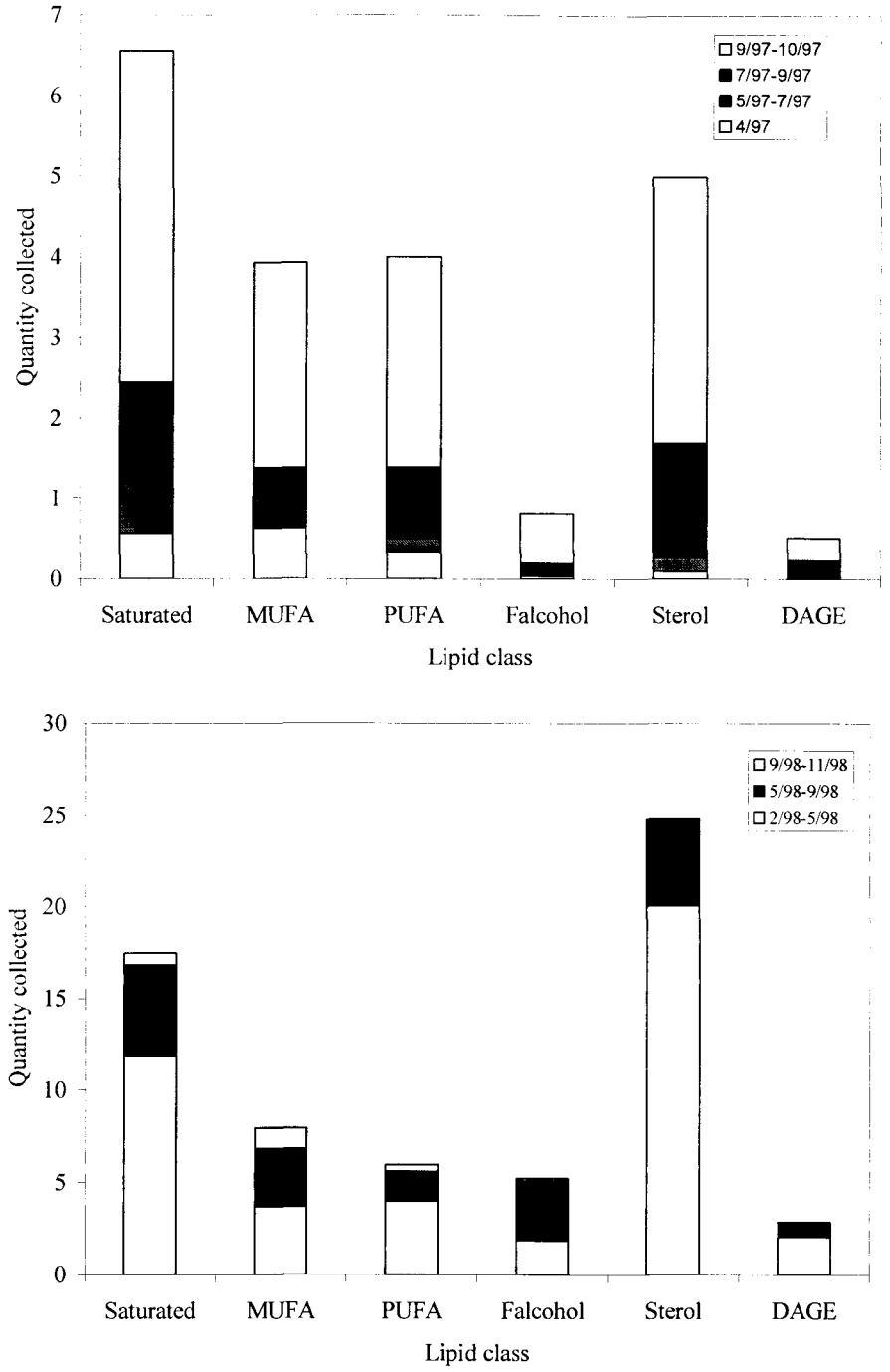
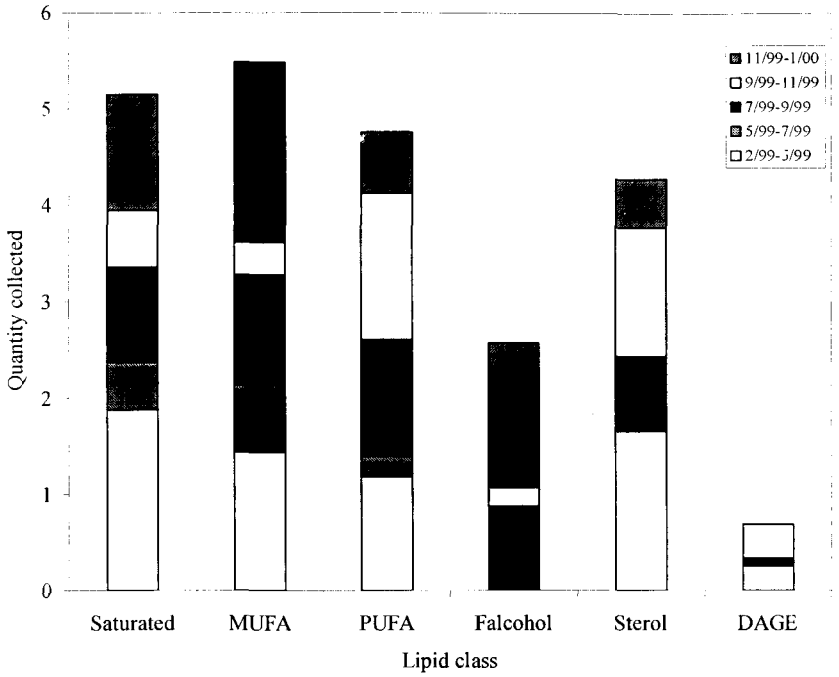


Figure 4.4a-b. The quantity of lipid (mmol lipid-C m^{-2} day^{-1}) collected during each sampling period, 1997-1998. SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, Falcohol = Fatty alcohol, DAGE = Diacylglycerol ether.



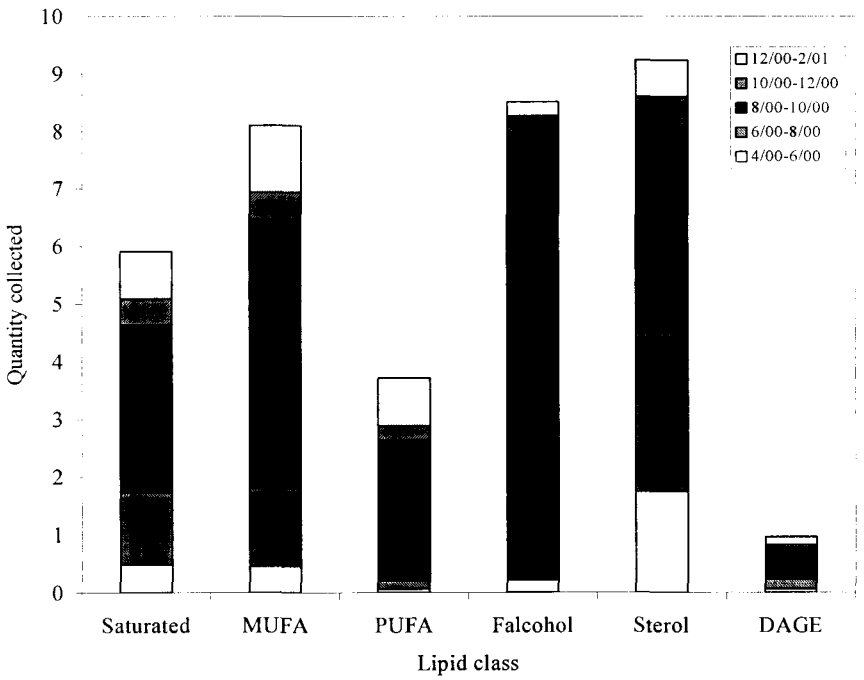


Figure 4.4c-d. The quantity of lipid (mmol lipid-C m^{-2} day^{-1}) collected during each sampling period, 1999-2000. SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, Falcohol = Fatty alcohol, DAGE = Diacylglycerol ether.

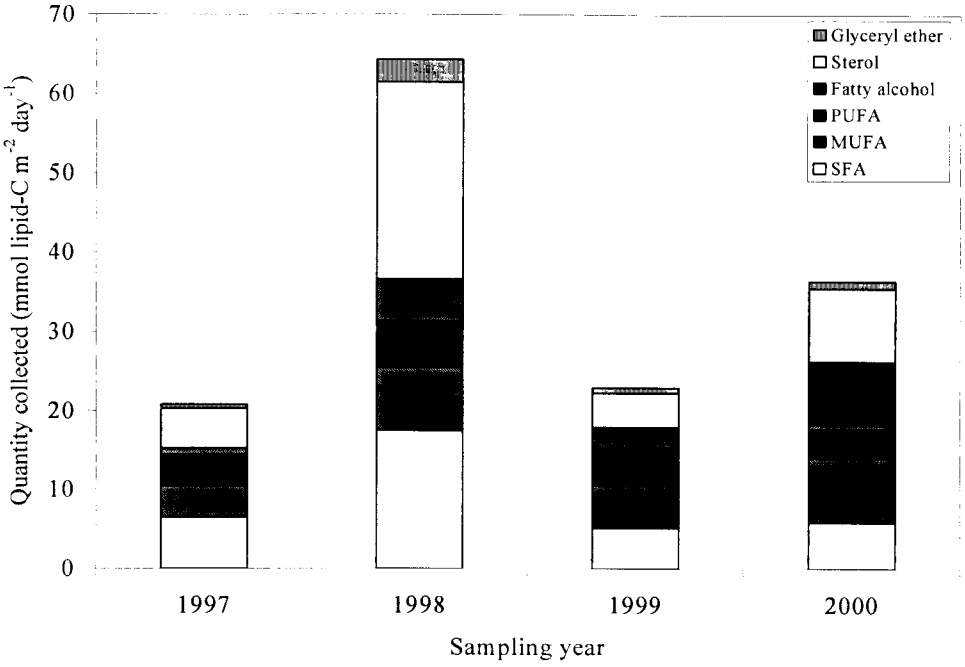


Figure 4.5. The quantity of lipid (mmol lipid-C m^{-2} day^{-1}) collected each year. SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid.

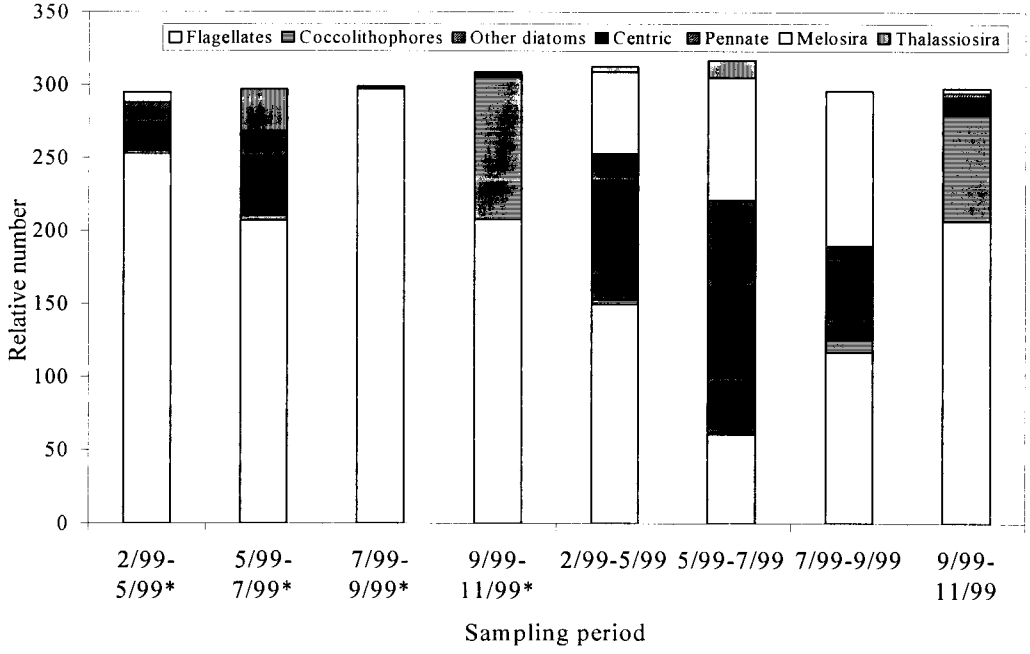


Figure 4.6. The relative number of phytoplankton counted for each sampling period. Phytoplankton from 2000 have not been counted.

Chapter 5.

Seasonal, interannual, and geographic variation in lipids of zooplankton from the southeastern Bering Sea*

Keywords: Lipids, fatty acids, fatty alcohols, sterols, zooplankton, Bering Sea

Abstract

The fatty acid, fatty alcohol and sterol compositions of lipids from zooplankton collected over the southeastern Bering Sea middle and outer shelf from 1997-2000 were determined. Zooplankton lipids differed from lipids found in sediment traps deployed at the same locations the zooplankton were collected. At the middle shelf site, more of the diatom fatty acid 16:1ω7 was found in trap samples than in zooplankton. This was probably due to a combination of the polyunsaturated fatty acid 20:5ω3 being retained in zooplankton bodies over sediment trap material, collection of diatoms in addition to fecal material by the sediment trap or fecal material having a composition intermediate between zooplankton and their food. Heterotrophic alteration of particles changes the lipid content of sediment trap matter, which usually contains much more cholesterol than zooplankton. Zooplankton lipids differed between winter and spring and among years in the amount of monounsaturated fatty acids and polyunsaturated fatty acids. These changes in lipids were reflected in the composition of lipids collected by the sediment traps. The most notable change in the herbivore fatty acid composition was the dramatic decrease in 16:1ω7 and 20:1ω9

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between February and April samples at both middle and outer shelf sites, resulting from zooplankton mobilization of wax esters and triacylglycerols for egg production. An unusually high abundance of the polyunsaturated fatty acid 22:6ω3, derived from prymensiophytes, was found in winter and spring 1998 *Calanus* spp. and *Sagitta* spp. samples, resulting from the coccolithophorid bloom in fall 1997.

5.1. Introduction

Fatty acids and other lipids have been utilized in a number of studies to provide information about ecosystems and the organisms that live within them (Virtue *et al.*, 1997; Falk-Peterson *et al.*, 1999). Specifically, lipids sollected by sediment traps have been used to compare production between sites and years (Wakeham *et al.*, 1982, 1997), and to reveal which organisms produce sinking particles at certain depths, how labile material is decomposed or transformed throughout the water column, how heterotrophs alter particles produced in the euphotic zone, and how these processes change seasonally (Wakeham and Canuel, 1986; Wakeham *et al.*, 1997). Since the seasonal and interannual variability in planktonic lipids are not known in most regions of the ocean, full application of this approach requires investigation of both plankton and sediment trap lipid composition.

Zooplankton lipids include polar, cell membrane lipids such as phospholipids, glycoplipids and sphingolipids, and storage lipids such as wax esters and triacylglycerols. Phospholipids, in particular, consist of a phosphoric acid esterified to two fatty acids. Typical fatty acids in phospholipids are polyunsaturated fatty acids like 20:5ω3 and 22:6ω3. Neutral lipids, such as triacylglycerols and wax esters are alcohols esterified to fatty acids. For example a triacylglycerol is a glycerol backbone esterified to three fatty acids. Wax esters have a one-to-one fatty alcohol:fatty acid ratio. Zooplankton, especially those exposed to colder sea surface temperatures and high food variability with starvation-like conditions in winter, use these neutrals lipids for energy storage, buoyancy and reproduction (Virtue *et al.*, 1997, Falk-Petersen *et*

al., 2000). Other important neutral lipids include sterols. Since copepods do not synthesize cholesterol, phytosterols in marine plants are the source, but they are modified by zooplankton. Other animals biosynthesize more specialized lipids, such as alkyldiacylglycerol ethers. Many of these lipid compounds, specifically the fatty acids, are unique to certain categories of organisms or occur in distinctive patterns. This property of lipids is what makes them useful in studying food web dynamics.

The study area was located on the southeastern Bering Sea shelf (Figure 1.1). The shelf is divided into three distinct hydrographic regions (the inner, middle and outer shelves), which are separated from each other by density fronts (Kinder and Schumacher, 1981; Coachman, 1986). The regions differ in water column depth, depth profiles of temperature, salinity and nutrient concentration, and the plankton and animal species at higher trophic levels (Kinder and Schumacher, 1981; Coachman, 1986). The middle shelf (situated between the 50 m and 100 m isobaths) is often ice-covered during cold winters and ice-free during mild winters. Increased solar radiation, warm temperatures, and water column stability trigger an ice-edge phytoplankton bloom as ice recedes in mid- to late-April (Niebauer *et al.*, 1995). Open water blooms during warm years usually begin in May. The outer shelf is located between the 100 m and 150 m isobaths. The outer shelf rarely has sea ice or ice-edge blooms; an open water mixed diatom bloom generally occurs there.

This chapter examines fatty acid and neutral lipid composition of zooplankton from two sites over the Bering Sea shelf over a four-year period, from 1997-2000. Sediment trap data from the same sites are used to compare lipid profiles of the animals

and sinking particles collected during the same time period. Comparison of the two data sets has yielded information about phytoplankton production, zooplankton production, lipid assimilation efficiency of zooplankton, and heterotrophic alteration of particle composition.

5.2 Methods

5.2.1. Collection

Zooplankton were sampled during cruises in the southeast Bering Sea from 1997-2000 (Table 5.1). Zooplankton were collected with oblique plankton tows using 333 μm and 153 μm or 53 μm CalVET mesh nets. Once aboard ship, zooplankton were separated by species, placed in glass vials and frozen at -20° C until ready for analysis.

5.2.2. Lipid extraction

Lipids were extracted from zooplankton using the Bligh and Dyer modified method for smaller sample sizes. The number of zooplankton used for each extraction varied, depending upon the animal and the time of year. For instance, for extractions involving 1-cm-long euphausiids (*Thysanoessa raschi* or *T. inermis*), lipids from two animals were extracted. For smaller, yet often lipid-rich copepods, between three and six specimens were used. Zooplankton were placed in precombusted test tubes (baked at 460° C for 8 hours) to which 0.625 ml of 2:1 methanol:chloroform was added. Using a Teflon®-coated pulverizing instrument, lipids were extracted from plankton tissue by mashing the plants or animals for one minute. Following this procedure,

0.625 ml chloroform was added to the mixture. The plankton were pulverized again for 30 seconds. Then 0.625 ml of glass distilled water was added and samples were mashed once more, after which a two-phase aqueous/organic system was formed, with total lipids being present in the bottom organic layer. The total solvent extracts were stored at -40° C until further analysis.

5.2.3. Saponification and silylation

A 200-µl aliquot of each total solvent extract was transferred to a baked test tube. Total solvent extracts were evaporated to dryness using a centrifugal evaporator. One ml of 5% w/v KOH in 80/20 methanol/water was added to each TSE aliquot in order to saponify the lipids. Saponification severs ester linkages: cholesterol esters (cholesterol esterified to fatty acids), wax esters (fatty alcohols esterified to fatty acids) and triacylglycerols (glycerol esterified to fatty acids) are broken up into their neutral lipid and fatty acid components. After methanolic KOH addition, the pH of the solutions was measured to verify a pH of 13. Solutions were heated at 60° C for 3 hours using a heating block. After samples cooled, 1 ml glass distilled water and then 1 ml 4:1 hexane:chloroform was added to each. Samples were vortex-mixed for 1 minute and allowed to settle into two phases: an upper organic phase containing saponifiable, neutral lipids, and a lower, aqueous phase containing fatty acids. A baked, glass Pasteur pipette was used to transfer the upper, organic fraction into a separate test tube. The contents were evaporated to dryness. Another 1 ml of the hexane: chloroform solvent was added to the sample. The second organic layer was removed. Then, the vortex/centrifugal evaporator procedure was repeated, leaving

behind the saponified, neutral lipid fraction, to which sterols, fatty alcohols (including the chlorophyll derivative, phytol) and hydrocarbons belong.

Finally, neutral lipids were derivatized using 25 µl BSTFA. The derivatizing agent forms volatile trimethylsilyl esters of neutral lipids, preparing them for GC (gas chromatographic) analysis. Samples were made up to a 1 ml volume with hexane:chloroform. Cholestane was used as an internal GC standard.

5.2.4. Fatty acid extraction and methylation

Approximately one-third ml of concentrated HCl was added to the aqueous layer remaining after the neutral lipid extraction step to lower the pH to 2. Then, 1 ml 4:1 hexane:chloroform was added to the aqueous layer , and the solution was vortex-mixed. A glass Pasteur pipette was used to transfer the organic fatty acid-containing layer to an awaiting, pre-combusted glass test tube. The solvent was evaporated to dryness, leaving the fatty acid portion behind. Another 1 ml hexane:chloroform was added to the aqueous layer and the solution mixed. A second organic layer was pipetted to the test tube, and the solvent evaporated, leaving fatty acids behind.

The fatty acids were then methylated, forming volatile fatty acid methyl esters in preparation for GC analysis. The procedure was as follows: One ml of a methylation solution (10:1:1; methanol, hydrochloric acid, chloroform) was added to the dry fatty acids. The fatty acid/methylation solution was then heated in a heating block at 100° C for one hour. After samples cooled, first 1 ml glass distilled water and then 1 ml 4:1 hexane:chloroform were added to each test tube. Samples were mixed for 1 minute using a vortex mixer and then allowed to settle into two phases: the

bottom aqueous layer and the top, organic phase. A baked, Pasteur pipette was used to transfer the upper, organic fraction into a separate test tube. The contents were evaporated to dryness. Another 1 ml of the hexane:chloroform solvent was added to the sample. The second organic layer was removed to the test tube. Then, the mixing and evaporation procedure was repeated. Each sample was made up to volume with the 4:1 hexane:chloroform solution. Methyl nonoanate was used as an internal standard for GC.

5.2.5. Gas chromatography

TMS derivatives and fatty acid methyl esters were analyzed by GC-MS (gas chromatography-mass spectrometry) using a Hewlett-Packard 5890 Series II Plus Gas Chromatograph. A 30 m X 0.25mm inner diameter capillary column with splitless injection was used with helium as the carrier gas. Injection temperature was 250° C and detector transfer line temperature was 300° C for fatty acids. Initial oven temperature was held at 180° C for two minutes then ramped at a rate of 2° C/min to 250° C. The flow rate was 1 ml/min. For sterol and fatty alcohol derivatives, injection temperature was 250° C and detector temperature was 290° C. Initial oven temperature was held at 180° C for four minutes, and then ramped at 3° C/min to 310° C.

5.2.6. Statistics

Analysis of variance was used to test for differences between sediment trap and zooplankton fatty acids and fatty alcohols, and interannual and seasonal differences between specific zooplankton fatty acids and fatty alcohols. Tukey's honestly

significant difference post hoc test was used to compare means at the 95% C.I.; t-tests were also used to test for differences between mean abundances.

5.3 Results

5.3.1. Comparison of zooplankton and sediment trap lipids

The fatty acids and neutral lipids were analyzed in 166 samples, which included 10 different zooplankton species and a mixture of phytoplankton, mainly diatoms, collected during a bloom. The 16:1ω7, 20:5ω3 and 22:6ω3 content of animals was significantly different from that of sediment trap material. M2 zooplankton were higher in 20:5ω3, while M2 trap samples contained a greater abundance of 16:1ω7 (all years totaled) (Figure 5.1a,b). On a year by year basis, zooplankton had more 20:5ω3 than 16:1ω7 and 22:6ω3 in 1998 and 1999 (Fig. 5.2a). For M2 sediment trap samples, the concentrations of 16:1ω7 and 20:5ω3 were not significantly different, but both were greater than 22:6ω3. In 2000, the concentration of 16:1ω7 was significantly greater than 20:5ω3 (Figure 5.2b). Tukey's HSD post hoc test and Figure 5.3a show that the 16:1ω7 concentration was significantly lower in both zooplankton and trap samples in 1998, yet there was significantly more of this diatom fatty acid in trap samples than in animals. Conversely, there was a statistically significant higher concentration of 20:5ω3 and 22:6ω3 in animals over sediment trap samples in 1998 (Fig. 5.3b-Fig. 5.3c). In addition to this, the 20:5ω3 and 22: ω3 concentrations were significantly lower in animals in 2000.

For fatty alcohols in animals, 20:1ω9 was the dominant fatty alcohol in 1997 and especially during the warm years, 1998 and 2000. The Tukey test showed no interannual variability for 20:1ω9, however. The shorter-chained 16:0 alcohol was dominant during the cold year 1999. The abundance of 16:0 increased steadily from 1997 to 1999 and decreased in 2000. A statistically significant difference was found between 1997 (lowest) and 1999 (highest) abundances (Fig. 5.4). Contrary to 16:0, the fatty alcohol 22:1ω11 decreased from 1997 to 1999. The statistical difference between the 1997 abundance and 1999 and 2000 abundances was significant. The phytol abundance decreased during the warm years, but this trend in phytol between years was not significant.

5.3.2. Fatty acid and fatty alcohol changes from winter to spring at M2 and M3

Over the four-year period, February (and June in 1997) was the month in which animals recorded the highest mean percentage of MUFA, in particular the diatom indicator, 16:ω7 (10.8%) and the lowest percentage of PUFA, mainly 20:5ω3 (10%) (Fig. 5.5a). By April and May, zooplankton were depleted in 16:1ω7 (3.6% and 5.3%, respectively) and rich in 20:5ω3 (14.2% and 16.7% respectively). Tukey's HSD post hoc test showed that 16:1ω7 abundance decreased significantly between February and April. At both sites M2 and M3, the diatom fatty acid showed a statistically significant decrease from February to April (Fig. 5.5a) A similar trend was noted in the fatty acid 20:1ω9; the February abundance decreased in April at both sites, which was

statistically significant (Fig. 5.5b). The trend was opposite for 20:5ω3, which increased in concentration between winter and spring at both sites (Fig. 5.5b).

The Tukey test was performed to detect significant changes in the fatty alcohol content of calanoid copepods *Calanus marshallae* and *Neocalanus* spp., since they are the dominant producers of wax esters containing long-chain alcohols. The post hoc test showed that no winter to spring difference in the relative abundance of 20:1ω9 existed. Conversely, a decrease in 22:1ω11 was statistically significant (Fig. 5.6). A significant increase was found for 16:0 between February and April. No significant change was found for phytol, however Phytol, which is a major fatty alcohol in euphausiid wax esters, is present in these zooplankton for at least half of the year. Its presence in both *T. raschi* and *T. inermis* showed that, in the Bering Sea ecosystem, both species may be detritivores. According to Falk-Petersen (1999), the lower abundance of phytol in *T. inermis*, especially during spring, differentiates them as herbivores from *T. raschi*, the more omnivorous species. A t-test performed on the phytol abundance in the two species did show a significant difference between them in the Bering Sea. *T. inermis,* the more herbivorous euphausiid, contains a high percentage of 14:0 and 16:0 fatty alcohols in its wax esters. The difference between *T. inermis* and *T. raschi* was significant for 14:0 but not 16:0.

5.3.3. Middle shelf versus outer shelf

Like middle shelf sediment trap samples, the 16:1ω7 fatty acid was lower in M3 samples during 1998 than during 1999, although its abundance was never as high as on the middle shelf (Figure 5.7). Conversely, the outer shelf trap was higher in

20:5ω3 in 1998, and the middle shelf trap was higher in this PUFA in1999. Outer shelf animals had a smaller abundance of 16:ω7 and 20:5ω3 than did middle shelf animals (Figure 5.8).

5.3.4. Unusual fatty acids

Chaeognaths had signature fatty acids not typically found in other organisms on the shelf. They biosynthesize 16:1ω5 and 18:1ω5, which no other organisms produce, except that 18:1ω5 was detected in *Calanus* in 1998. Also a longer chain fatty acid, 24:1, was detected in 1998 *Calanus* and in chaetognath samples.

The pteropod *Clione limacina* biosynthesizes several unusual odd-chained fatty acids such as 15:0, 15:1, 17:0, 17:1 (two isomers) and 19:1 (two isomers). These fatty acids are part of its alkyldiacylglyeryl ethers (DAGE). The organism also contains the unusual fatty alcohol, 15:0.

5.4. Discussion

5.4.1. Comparison of zooplankton and sediment trap lipids

Figure 5.1 shows that 20:5ω3 was twice as abundant as 16:1ω7 in zooplankton, but that the latter was one and one-half times as abundant as 20:5ω3 in trap samples for the four years totaled. One possible explanation for the difference is that the zooplankton preferentially assimilate PUFA. If this is the case, percentage-wise, zooplankton retained almost one and one-half times more 20:5ω3 than they excreted as sinking particles to be collected by the traps. They assimilated five times more 22:6ω3

than they excreted. They also excreted twice as much 16:1ω7 as they utilized, for instance, for incorporation into neutral lipids. PUFA such as 20:5ω3 and 22:6ω3 are a high percentage of zooplankton cellular membrane phospholipids (Falk-Petersen, 2000), especially in areas where sea surface temperatures are colder. The low melting points of these compounds enable organisms to maintain cell membrane fluidity at lower temperatures (Pruitt, 1990). The MUFA 16:1ω7, a diatom indicator, is often part of zooplankton neutral lipids, such as triacylglycerol and wax esters. Falk-Petersen *et al.* (2000) found that *T. inermis* collected from Svalbard contained 16:1ω7 as nearly one-third of its triacylglycerol fatty acid in July and 21% in September. The fatty acid was also 14% of its wax ester in July. The findings for *T. raschi* were similar. The MUFA accounted for 23% and 15% of triacylglycerol fatty acid in July and September, respectively. It also was 45% of the wax ester fatty acid. Conversely, the PUFA 20:5ω3 and 22:6ω3 were not above 4% in either compound class or either organism, but they did comprise a substantial portion of polar lipids in both euphausiids (between 26% and 30%).

Another possible reason that the 16:1ω7 fatty acid is higher in trap samples is that the trap samples consist partly of diatoms that sank without being grazed. Diatom samples were high in both fatty acids, although they were higher in the MUFA (18.5%) than the PUFA (14.8%). A related explanation is that the fatty acid composition of fecal material is likely to be intermediate between that of zooplankton and their food.

A major difference between lipids detected in the sediment trap samples and lipids detected in zooplankton was the presence of a high quantity of sterols, mainly cholesterol in the sediment trap samples. Very little cholesterol was found in zooplankton. In most zooplankton samples, cholesterol was below the detection level, especially in comparison to the quantity of fatty alcohols found in animal tissue. Cholesterol and 24-methylcholesta-5,22-dien-3ß-ol (brassicasterol, once termed diatomsterol) were detected as major neutral lipids in chaetognath and fish samples taken from 1998. Harvey *et al.* (1987) showed that the greater the food supply, the greater the quantity of cholesterol and other sterols that zooplankton excrete. Often zooplankton demethylate sterols and excrete the cholesterol.

5.4.2. Seasonal variability

Figure 5.5a and Figure 5.5b show the mean monthly changes in fatty acids 16:1ω7, 20:5ω3 and 20:1ω9 at sites M2 and M3. Overall, the MUFA concentration decreased in April to less than one-half its February value, while the 20:5ω3 abundance increased. The decrease in fatty acids that are major constituents of zooplankton wax esters and triacylglycerols show mobilization of lipids for egg production in April (Sargent and Henderson, 1986). All zooplankton show this trend, both herbivores and their predators. In addition, 20:1ω9 also shows a significant decrease in pre-bloom spring abundances.

Figure 5.6 shows a seasonal decrease in calanoid copepod wax ester fatty alcohols 20:1ω9 and 22:1ω11, mobilized for spawning and a commensurate increase in16:0 and phytol. By May, the monoenoic alcohols increase while the others

decrease. Phytol is a side-chain to the pigment chlorophyll. Its presence in animals usually represents detrital feeding (Falk-Petersen *et al.*, 2000); therefore its concentration decreases with the spring bloom. The saturated fatty alcohol 16:0 increases in May but decreases to one-third of its May concentration by June, indicating less need for lower melting point alcohols when sea surface temperatures increase.

In outer shelf sediment trap samples, similar to the middle shelf site, 20:5ω3 was a higher percent of the fatty acids than was the diatom indicator 16:1ω7 in 1998 and 1999 (Figure 5.7). The PUFA were almost eight times as abundant as the MUFA in 1998 and four times as abundant in 1999. The fatty acid 16:1ω7 became a higher percentage of the total fatty acids in 1999, as it was only 2% of the total fatty acids in 1998. This trend is similar to that of the middle shelf site, in which 16:1ω7 was an unusually small portion compared to the other years. The zooplankton collected from the outer shelf contained only one-third as much 16:1ω7 as did animals collected from the middle shelf site in 1997 (Figure 5.8). Animals from the middle shelf had higher abundances in the PUFA 20:5ω3 than did animals from the outer shelf, but interannual trends were the same. For instance, 1998 had the highest concentration of 20:5ω3, followed by 1999, 1997 and 2000. A possible reason that outer shelf animals, and therefore trap samples, have less 16:1ω7 and 20:5ω3 is that these are both probably derived from diatoms. Zooplankton over the middle shelf may have a less varied diet than zooplankton from the outer shelf, or perhaps the species of diatoms differ,

producing different fatty acids. For instance, a large variety of diatoms have been identified in sediment trap samples from the middle shelf. Outer shelf sediment trap samples have more fecal pellets and a large number of unidentified flagellates. The major diatoms present over the outer shelf during spring cruises have been *Chaetoceros* spp. Fatty acid profiles of these diatoms show that although they possess a high abundance of 16:1ω7, they also have a high proportion of 20:1ω9, and this ramifies up the food chain to the herbivores *N. plumchrus* and *N. cristatus*. In addition, these copepods biosynthesize this fatty acid and transform it into 20:1ω9 fatty alcohol. A third reason that the outer shelf is lower in the diatom indictor is that the herbivore *N. cristatus* reduces it to 16:1ω7 fatty alcohol. Other copepods that have 16:0 and 16:1ω7 among their fatty alcohols always have a higher abundance of 16:0. However, *N. cristatus* always has a higher abundance of 16:1ω7 alcohol than 16:0.

5.4.3. Interannual variability

The zooplankton fatty acids (Figure 5.2) were similar among years in all but one respect. In the 2000 animals, 16:1ω7 had a higher mean relative abundance than 20:5ω3. The sediment trap samples from 2000 reflected this trend, showing a greater difference between the MUFA and PUFA than in previous years. This occurred at both middle and outer shelf sites, and was evident in animals normally high in PUFA, such as the chaetognaths, which showed a two-fold drop in 20:5ω3 (10%) abundance from the previous three years, which were all similar. This significant decrease in polar lipids in organisms across the shelf could indicate that diatoms produced these

compounds in smaller quantities during the previous fall's diatom bloom. It could also reflect a change in the physical ecosystem. According to Hunt *et al.* (2002), 2000 was an unusually warm year in the Bering Sea. Since PUFA are primarily polar lipids intended to keep membranes fluid, warmer temperatures could cause organism to produce fewer PUFA. In essence, fewer PUFA in marine organisms could indicate warmer climate.

If it is supposed that zooplankton were metabolizing their storage lipids during winter's low food supply, this could affect the PUFA abundance. In a 130-day starvation experiment (Virtue *et al.*, 1997) involving the Antarctic euphausiid *Euphausia superba,* polar lipid levels decreased significantly, but only between day 1 and day 5. Late winter 1999 and early winter 2000 recorded warmer than average sea surface temperatures, so starvation and excess lipid metabolism probably did not deplete the PUFA abundance.

A massive coccolithophorid bloom covered the shelf in fall 1999. There was also one in fall 1997. A difference between the February 1998 organisms collected and the 2000 organisms is that coccolithophorid indicators such as 22:6ω3 (Conte *et al.*, 1994a) were incorporated into 1998 organisms but not 2000 organisms. A possible explanation is that, in addition to the coccolithophorid *Emiliania huxleyi*, the zooplankton were feeding on dinoflagellates or *Phaeocystis pouchetti*, which are both high in 22:6ω3 (Conte *et al.*, 1994a,b). Another factor could be that the zooplankton were continuously feeding into 1998, if the fall 1997 coccolithophorid bloom persisted well into 1998. For example, zooplankton collected from the northern-most middle

shelf sampling site were high in 22:6ω3 in April and May. Satellite images show the bloom had moved further north in 1998. The animals collected from the more southerly trap site were high in 22:6ω3 in February and May, but not in April. This suggests that they were continuing to feed on the source of the PUFA the following spring. However, neither dinoflagellates nor *Phaeocystis* were numerous in the microscopic analysis of trap samples. Whatever the source of 22:6ω3, it did not exist in late 1999 or early 2000. Since marine animals cannot biosynthesize PUFA in great quantities, they are strictly dependent on diet for these compounds. The low PUFA also affected *C. marshallae* predator *Sagitta* spp., which was low in PUFA. Zooplankton can, however, biosynthesize monounsaturated fatty acids, which were plentiful in 2000. Many species recorded increases in 16:1ω7, its elongation product 18:1ω7 and 20:1ω9 concentrations.

Figure 5.3a shows that for all years, 16:1ω7 had its lowest mean relative abundance in 1998, in both animal and trap samples. This is most likely due to the fact that in animals in 1998, PUFA were dominant in the total fatty acids. The trap samples reflect that animals possessed a smaller quantity of 16:1ω7 to excrete, possibly due to smaller diatom production during the bloom and more coccolithophorids (as seen from the increase in 22:6ω3). They also could have been elongating dietary fatty acids to produce other monounsaturates such as 18:1ω7. The PUFA 20:5ω3 had its highest abundance in animals in 1998 (followed closely by 1999) and its highest relative abundance in the sediment trap in 1999. Animals were apparently assimilating the

PUFA to a greater extent in 1998, which was a warm year, but metabolizing or excreting more PUFA in 1999, a cold year. If zooplankton grow more slowly during colder years, assimilating less PUFA produced by phytoplankton and grazing a smaller portion of the bloom could be factors that affect the proportion of PUFA in their bodies. Assimilation efficiency, the ratio of the food assimilated to the food ingested, is affected by a suite of factors. The main factors are food quality, food quantity, and the age of the consumer. Food loss during feeding by copepods can be as much as 30% (Williams, 1981). According to Gaudy, assimilation efficiency in *Calanus helgolandicus* is between 12.4% and 61%. When the quantity of food is high, the assimilation efficiency is less. Since the maximum abundance of 20:5ω3 descended to the trap during the warm early fall, zooplankton were probably grazing heavily on high quality food, consuming larger quantities, but losing a high percentage in fecal material. The PUFA 22:6ω3 had the highest relative abundance in animals in 1998 and its highest abundance it trap samples in 1999. Its lowest abundance in trap samples was also in 1998. In 1998, the zooplankton were assimilating the polyunsaturated fatty acid, mostly likely due to warmer sea surface temperatures and high heat content of the water (Stabeno *et al.*, 1999), which increased assimilation efficiency. Although the overall 1999 animal abundance of 22:6ω3 was one-third that of 1998's, its sediment trap samples contained seven times more. So even though 22:6ω3 percentages were higher in animals than in trap samples, PUFA loss was greater in 1999 than in other years. Also, 20:5ω3 percentages are higher in animals than in traps, but 16:1ω7

percentages are higher in sediment trap samples than in animals, probably due to animal loss and direct diatom input.

Figure 5.4a shows the interannual changes in zooplankton fatty alcohols, 1997-2000. The16:0 abundance increased more than two-fold between warm year 1998 and cold year 1999. It had the highest abundance in zooplankton in 1999. According to Sargent and Henderson (1986), short-chained fatty alcohols have lower melting points than the longer-chained 20:1ω9 and 22:1ω11. Therefore, zooplankton were apparently replacing a portion of their wax ester fatty alcohols to compensate for climate variability. The fatty alcohol composition of sediment trap samples is shown in Figure 5.4b and Figure 5.4c. The quantities of fatty alcohols in 1997 and 1998 sediment trap samples were much less than those in 1999 and 2000. The fatty alcohol 20:1ω9 was dominant all years, except 2000. Since it is also the major fatty alcohol in zooplankton, it was lost in proportion to its production.

5.5 Summary and Conclusions

The lipids of macrozooplankton collected from middle and outer shelf sites of the southeastern Bering Sea, 1997-2000, were analyzed. Zooplankton contained a higher mean relative percent of ω3 polyunsaturated fatty acids (PUFA), such as 20:5ω3 (14%) and 22:6ω3 (5%), than 16:1ω7 (7%). On the other hand, the monounsaturated fatty acid (MUFA) 16:1ω7 (16%) was a greater portion of the fatty acids found in sediment trap material than were 20:5ω3 (10%) and 22:6ω3 (1%). Fatty alcohols,

especially 20:1ω9 and 22:1ω11, were the dominant neutral lipids detected in animals. Cholesterol, which was a dominant lipid in late 1997, early 1998, fall 1999 and spring 2000 in sediment trap samples, was only a trace constituent of neutral lipids in all zooplankton every year except 1998. This suggests that sediment traps collected substantial fecal material, since it is enriched in cholesterol over the zooplankton themselves.

Zooplankton fatty acid composition varied seasonally. The most notable change in the herbivore fatty acid composition was the dramatic decrease in 16:1ω7 and 20:1ω9 between February and April at both middle and outer shelf sites, resulting from zooplankton mobilization of wax esters and triacylglycerols for egg production. Carnivores whose main food source is herbivorous zooplankton experienced a concomitant drop in these fatty acids. Location (middle vs. outer shelf) also influenced the total fatty acid content. Species from the middle shelf had more PUFA than those from the outer shelf, especially herbivores such as *Neocalanus* spp., and tended to be higher in the saturated fatty acids 14:0, 16:0 and 18:0.

Interannual variability in fatty acids was also noted, especially in 1998 specimens. An unusually high abundance of the polyunsaturated fatty acid 22:6ω3, derived from prymensiophytes, was found in winter and spring 1998 *Calanus* spp. and *Sagitta* spp. samples, resulting from the coccolithophorid bloom in fall 1997.

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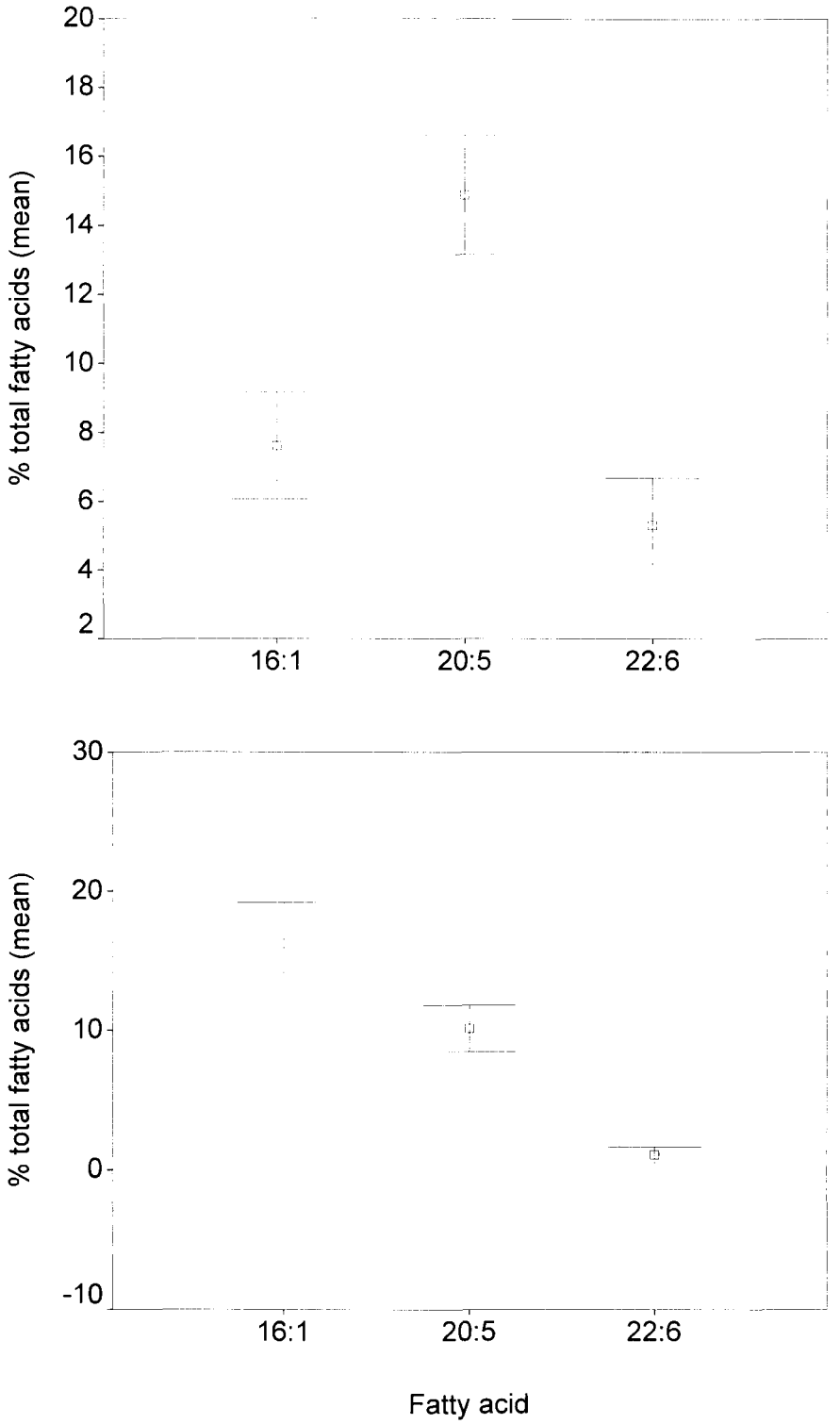
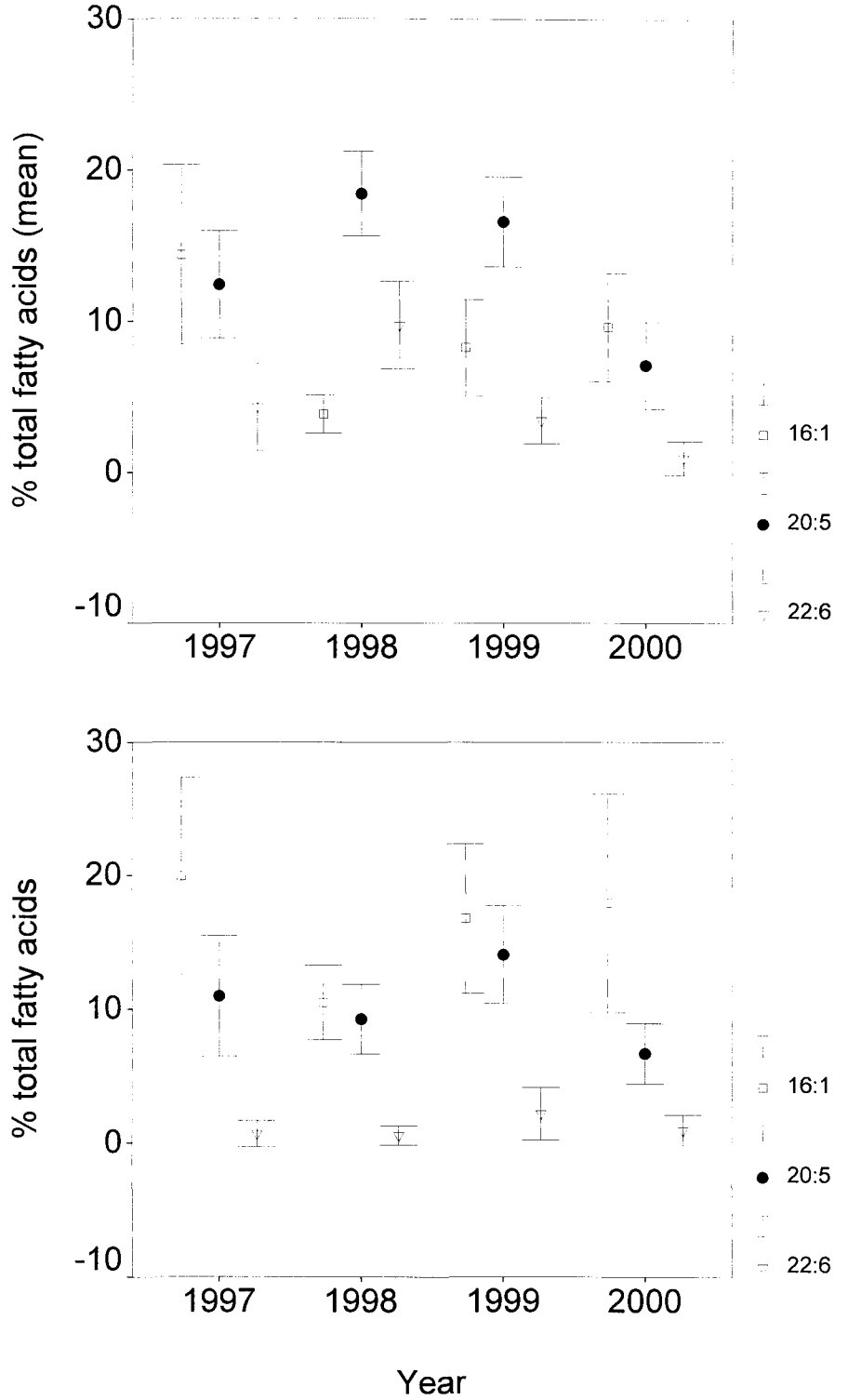
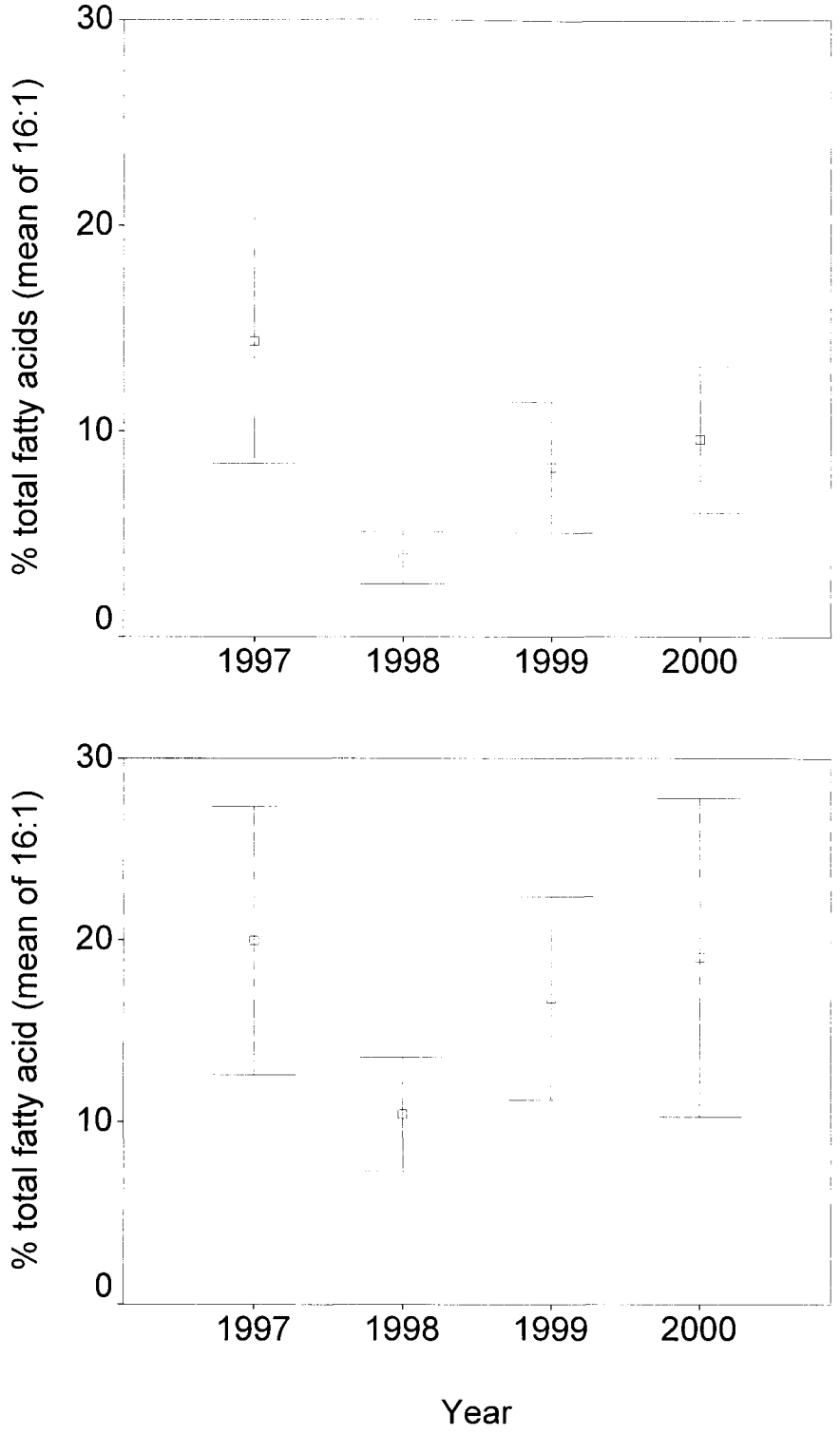


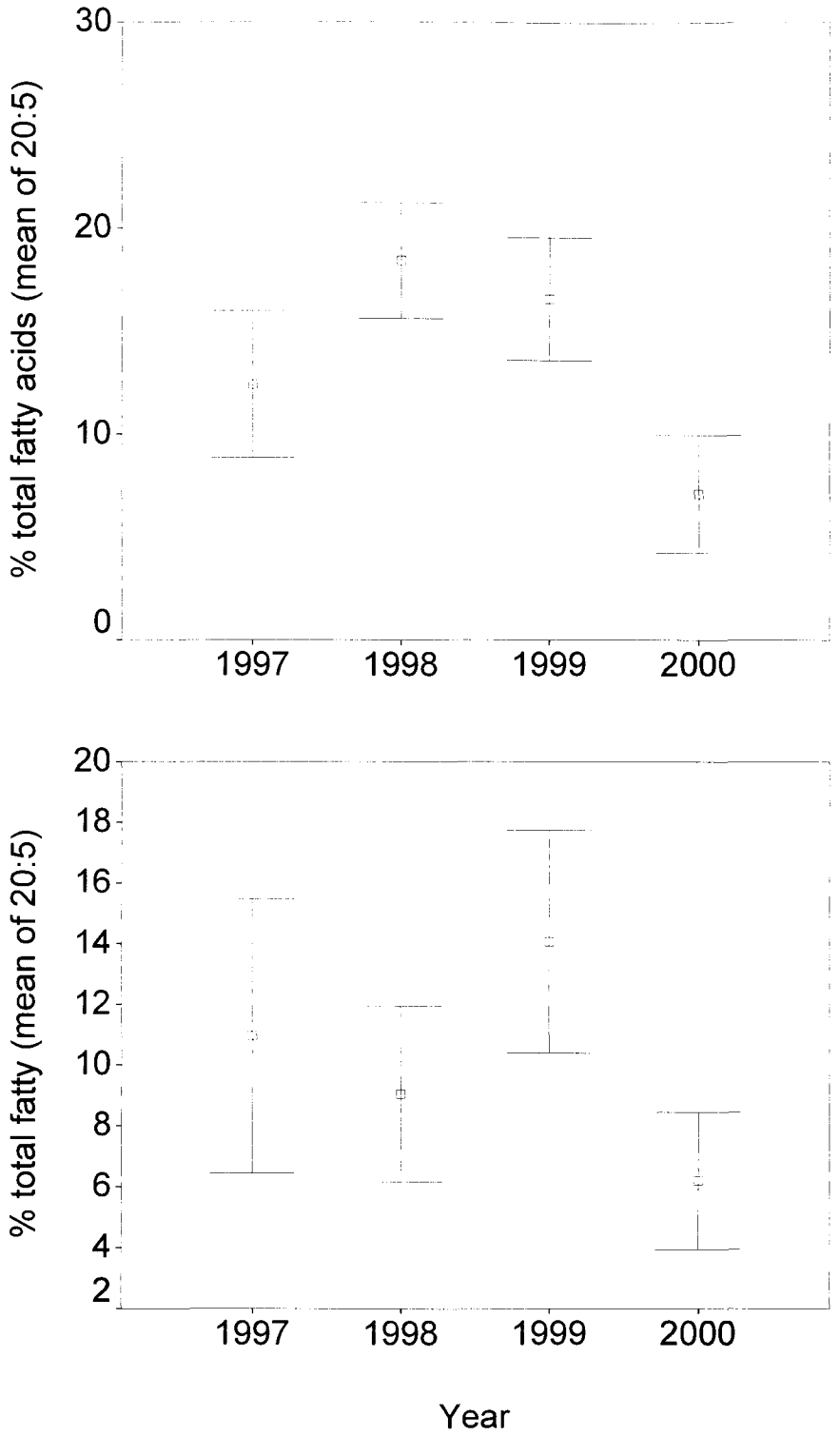
Figures 5.1a-b. Dominant fatty acids in zooplankton and sediment trap samples. The PUFA 20:5ω3 is twice as abundant in animals (top) as 16:1ω7, but 16:1ω7 is dominant in sediment trap samples. The PUFA 22:6ω3 is only a small portion of each.



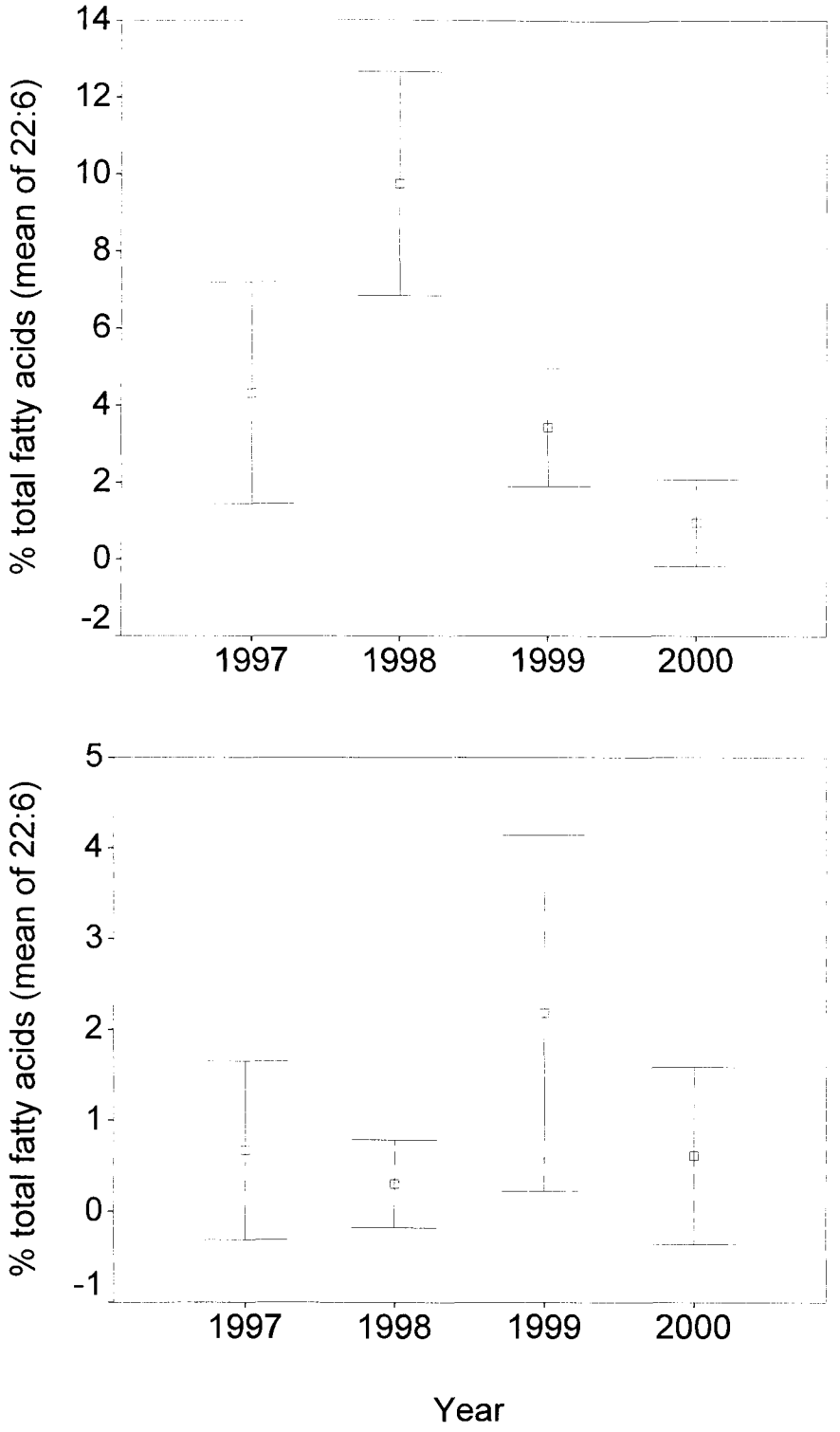
Figures 5.2a-b.Interannual changes in fatty acids of zooplankton (top) and sediment trap samples (bottom). Sediment trap tended to have more 16:1ω7, while animals have more PUFA.



Figures 5.3a. Interannual changes in the diatom indicator 16:1ω7 in animals (top) and in sediment trap samples (bottom). The lowest abundance of this fatty acid was in 1998.



Figures 5.3b. Interannual changes in 20:5ω3 in zooplankton (top) and in the sediment trap samples (bottom). Zooplankton contained more of the PUFA in 1998, but more was collected in the trap in 1999.



Figures 5.3c. Interannual changes in 22:6ω3 in zooplankton (top) and in sediment trap samples (bottom). The PUFA increased significantly in zooplankton in 1998, but had its lowest concentration in trap samples that year.

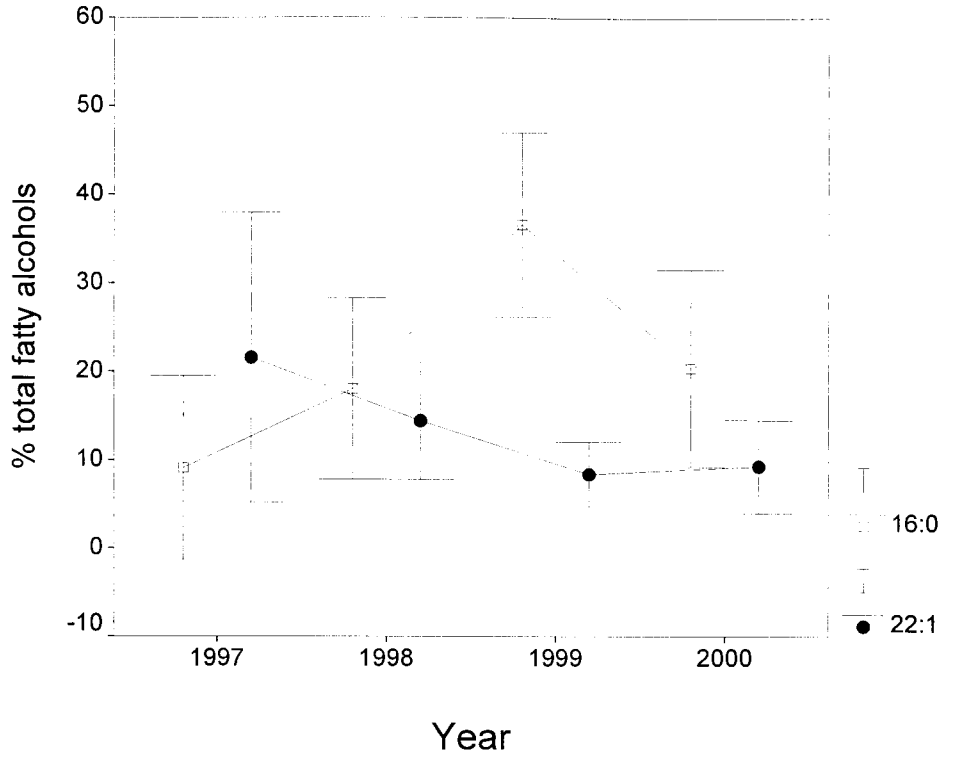
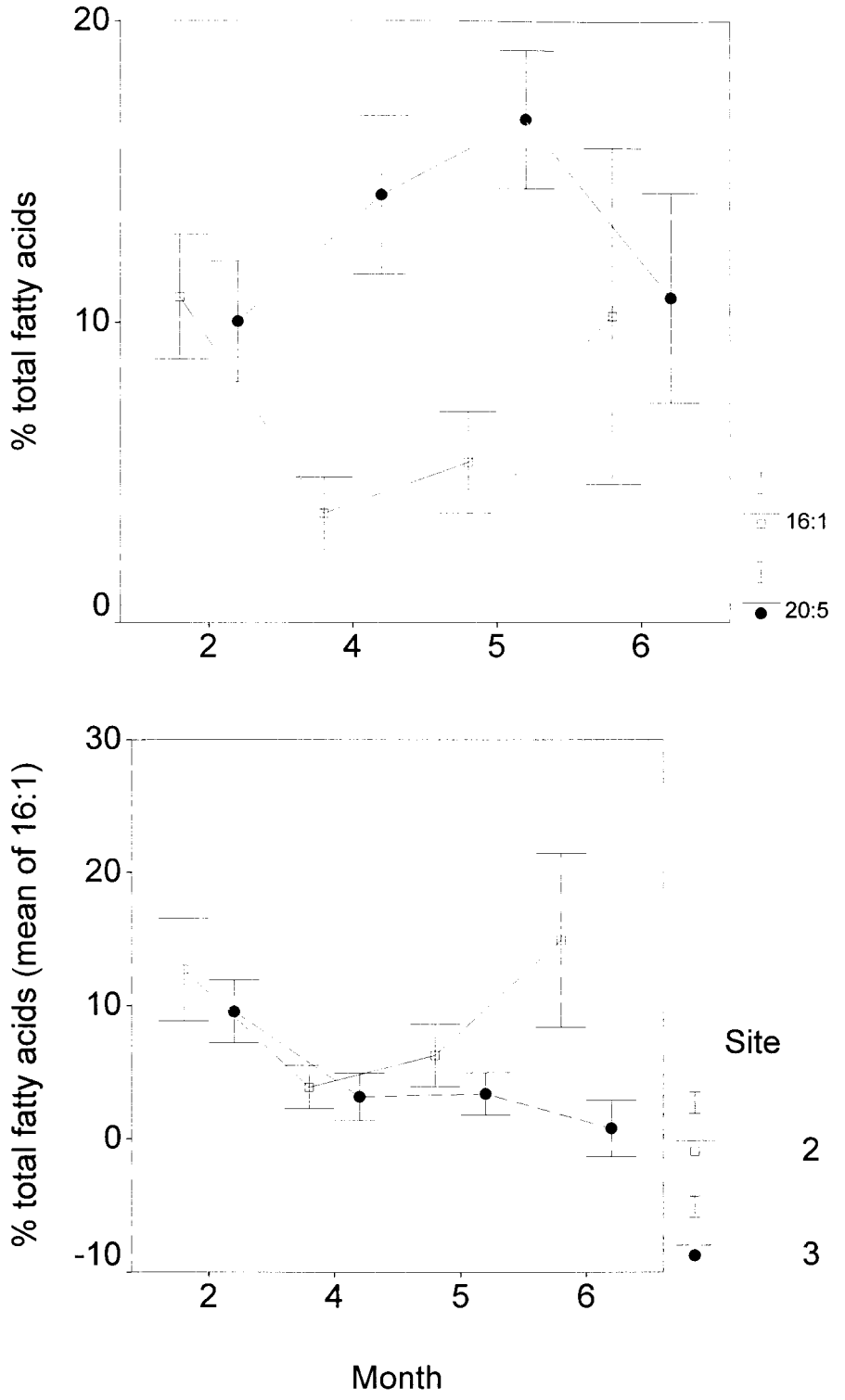
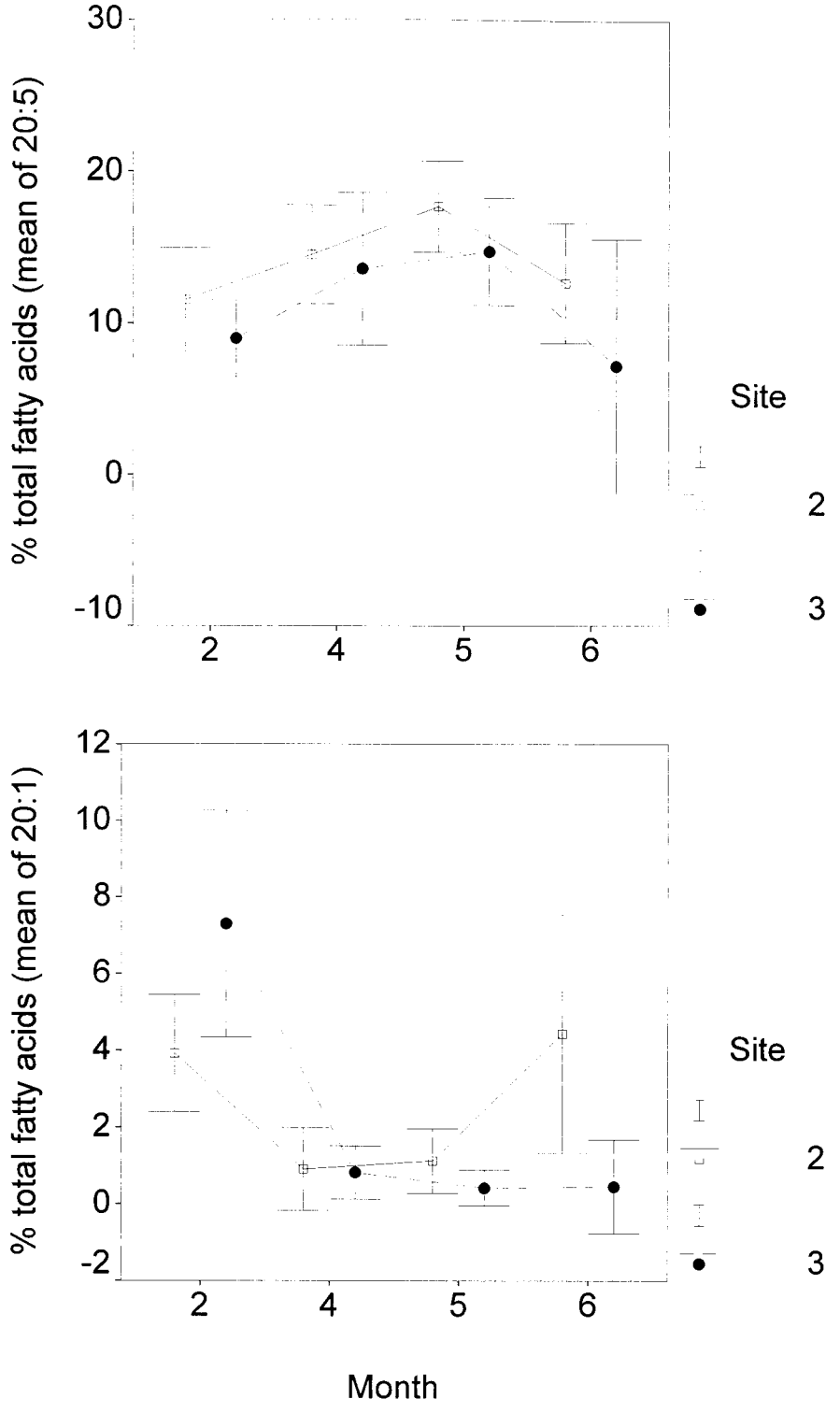


Figure 5.4. Interannual variability in fatty alcohols. During 1999, a year when ice advanced in May, 16:0 increased two-fold. The 22:1ω11 concentration steadily decreased.



Figures 5.5a-b. Winter to spring changes in fatty acids for all zooplankton (top) and in sites 2 and 3 zooplankton (bottom). The diatom indicator decreased at both sites. It then increased as site 2, but continued to decrease at site 3.



Figures 5.5c-d. Monthly changes in zooplankton 20:5:ω3 concentration (top) and 20:1ω9 concentration (bottom) at sites 2 and 3.

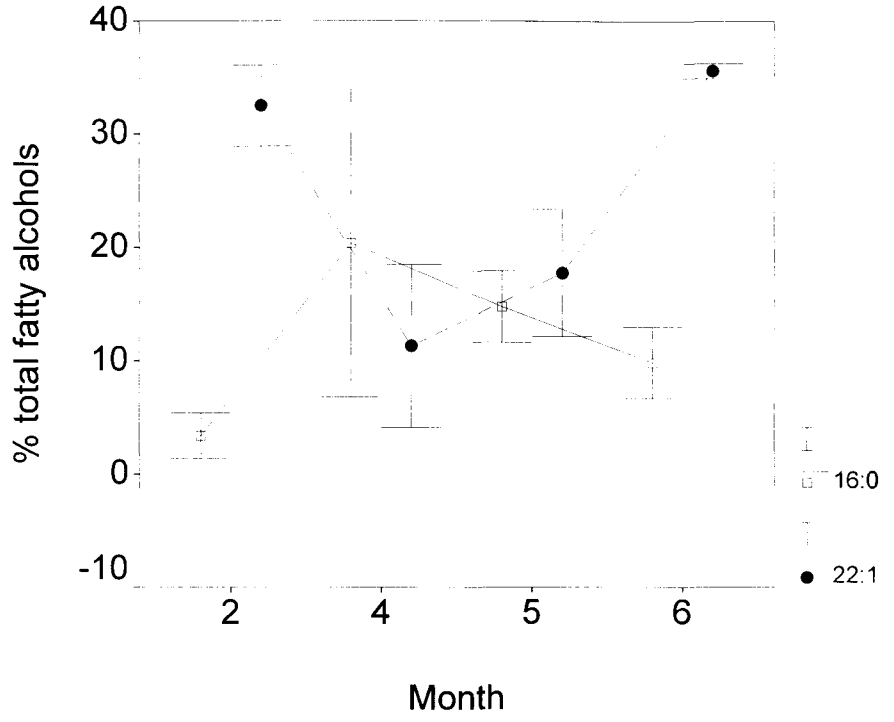
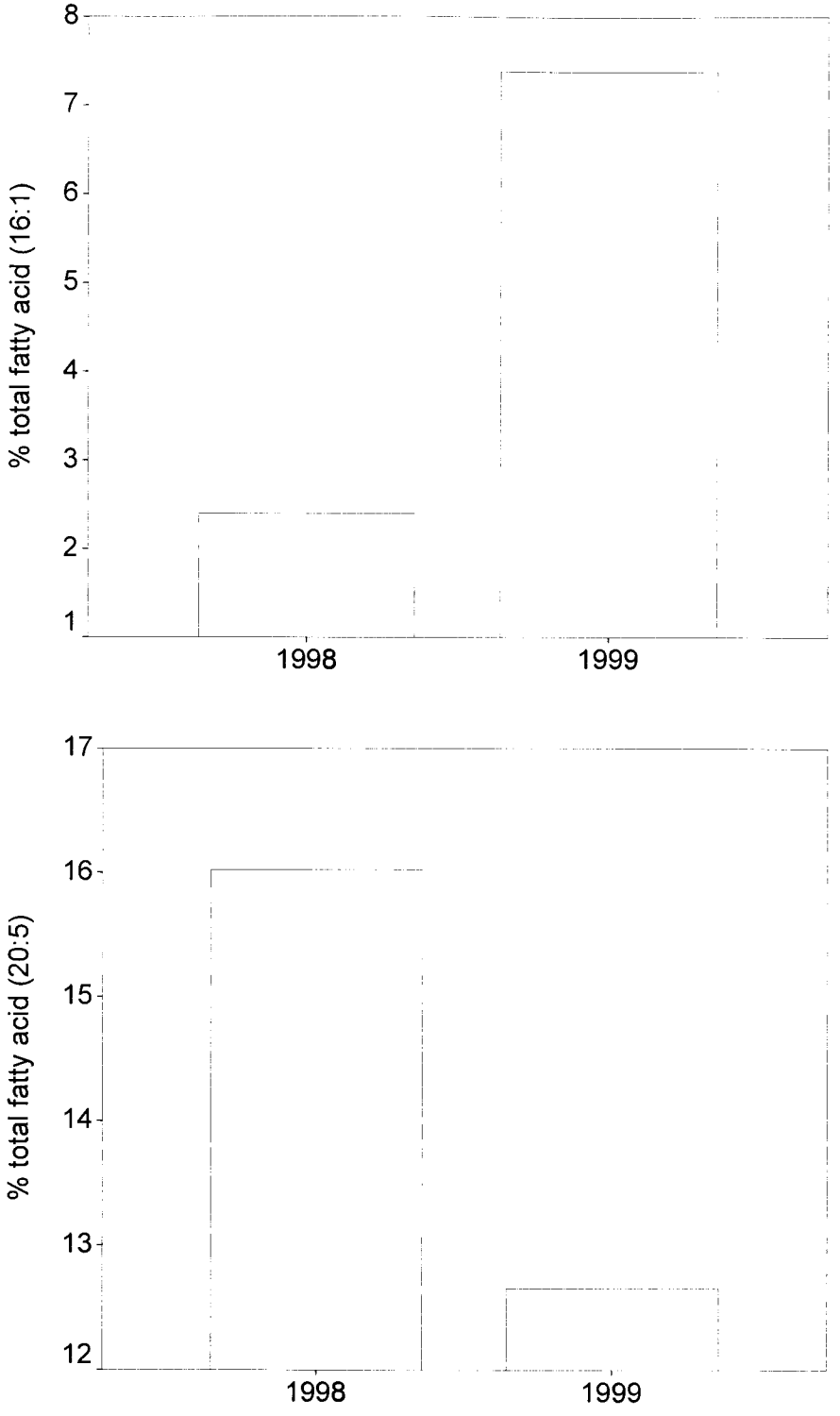
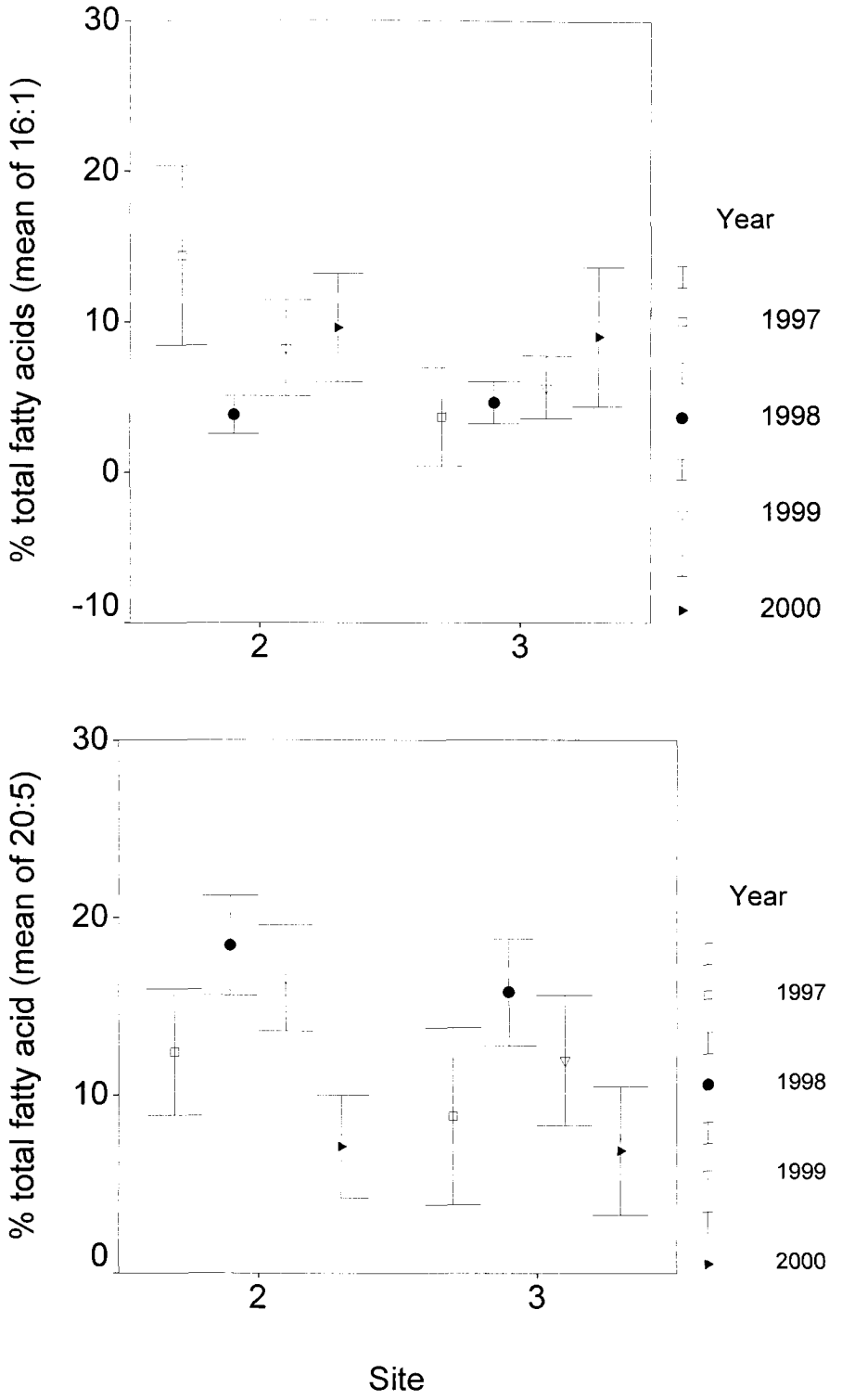


Figure 5.6. Monthly changes in fatty alcohol abundances in calanoid copepods. The 22:1ω11 concentration was highest in February and decreased significantly in April. Conversely, 16:0 had a greater abundance in April.



Figures 5.7a-b. Fatty acids in site M3 sediment trap samples. The fatty acid 16:1ω7 (top) had a low abundance in1998. The PUFA 20:5ω3 (bottom) was higher in 1998.



Figures 5.8a-b. Comparison of zooplankton fatty acids at site 2 and site 3, 16:1ω7 (top) and 20:5ω3 (bottom). Greater interannual changes in site 2 zooplankton fatty acids were exhibited. A decreasing trend in 20:5ω3 abundance is seen at both sites.

Table 5.1. Zooplankton collection dates

	SITE OF ZOOPLANKTON COLLECTION		
Year	M2	M3	M4
1997	February 18-26	February 1-26	Ice covered
	April 15-30	April 15-30	April 15-30
	June 10-27	June 10-27	June 10-27
1998	February 23,24	February 25,26	Ice covered
	April 15,16	April 26,27	April 17,18
	May 11,12	May 9,10	May 13, 14
1999	February* 7,8	February 6,7	Ice covered
	April 24,25	April 26	April 27
	May 2,3,8,9	May 6,7	May 4,5
2000	February 19,20	February 20,21	Ice covered
	April 23,24	April 26,27	April 25
	May 3,4	May 8,9	May 5,6

*The original mooring site was covered with ice. The sediment trap was deployed at a nearby site called M2a, which was ice-free. Zooplankton were collected at M2a site in February 1999.

Chapter 6.

Summary and conclusions

The results of this dissertation support the proposition that the southeastern Bering Sea ecosystem changes in response to interannual variability in weather. The organic matter collected in sediment traps from 1997 to 2000 showed that weather affects both primary and secondary production. Seasonal and interannual changes in stable isotopic composition of sinking particulate matter and zooplankton corresponded to changes in primary productivity and nutrient availability, while changes in fatty acids, fatty alcohols and sterols indicated changes in the food web, such as the extent of grazing of phytoplankton production by zooplankton. Comparing stable isotopes and lipids between the middle and outer shelf revealed differences in nutrient supply, primary production or primary producer species, timing of blooms and grazing, which were maintained despite interannual variability.

Specifically, isotopic data have shown large interannual, seasonal and geographic changes in $\delta^{15}N$ and $\delta^{13}C$ of sinking particles over the middle shelf (M2). Warm summer sea surface temperatures during the El Niño years 1997 and early 1998 were linked to greater $\delta^{15}N$ in the sinking particles and zooplankton. The $\delta^{15}N$ and the $\delta^{13}C$ values of suspended particulate matter, sediment trap material and zooplankton from the middle shelf were greater than those at the outer shelf site (M3). This pattern could be explained by greater primary productivity and a greater extent of nutrient depletion at the middle shelf site. Primary production by smaller phytoplankton cells

at M3 than at M2, and regeneration of isotopically heavy nitrogen from bottom sediment detritus at M2, are additional factors that could contribute to this spatial pattern. The quantities of material collected in early spring and late fall were greater than mid-summer collections, but spring blooms did not supply most of the organic matter collected by the traps. The material collected by sediment traps indicated that the productive period in the Bering Sea can extend from March through October, substantially longer than indicated by earlier studies. Whether this is because the productive season is actually longer in the late 1990s than the late 1970s, or simply because 1970s observations were predominantly during the April-August period, is uncertain.

Interannual changes in the lipids in sediment trap samples from the middle shelf support aspects of the Hunt *et al.* (2002) Oscillating Control Hypothesis and earlier models of the Bering Sea food web (Walsh and McRoy, 1986). Fatty acids indicative of primary producers were found during the spring of colder, ice-edge bloom years. This indicated less coupling between phytoplankton and zooplankton. On the other hand, the composition of lipids found during springtime of warm, open water bloom years indicated extensive zooplankton grazing of phytoplankton production. The fatty acid 16:ω7, an indicator of diatoms, was a higher percentage of total fatty acids during cold years, evidence that ice-edge blooms often sink ungrazed. Conversely, cholesterol, which was a dominant lipid in late 1997, early 1998, fall 1999 and spring 2000 in sediment trap samples, was only a trace constituent of neutral lipids in all

zooplankton during every year except 1998. This lipid is enriched in zooplankton fecal material, accounting for its presence in the trap samples.

Location affected the particulate matter collected by the sediment traps. The trap at M2 collected more organic carbon than the trap at M3 during both 1998 and 1999. Also, M2 sediment trap samples contained a higher percentage of diatom indicators. Location also influenced the fatty acid composition of zooplankton. Species from the middle shelf had more PUFA than those from the outer shelf, especially herbivores such as *Neocalanus* spp., which tended to be higher in the saturated fatty acids 14:0, 16:0 and 18:0. Overall, the geographic patterns are consistent with more grazing of primary production over the outer than the middle shelf.

Zooplankton fatty acid composition varied seasonally. There was a dramatic decrease in 16:1ω7 and 20:1ω9 between February and April for the samples collected at both the middle and outer shelf sites, resulting from zooplankton mobilization of wax esters and triacylglycerols for egg production. Carnivores whose main food source is herbivorous zooplankton experienced a concomitant drop in these fatty acids.

Interannual differences in zooplankton fatty acids were also noted, especially in 1998 specimens. An unusually high abundance of the polyunsaturated fatty acid 22:6ω3, derived from prymnesiophytes, was found in winter and spring 1998 *Calanus* spp. and *Sagitta* spp., resulting from the coccolithophorid bloom in fall 1997. Also, the diatom indicator, 16:1ω7, showed a significantly lower abundance in 1998 animals than in 1997, 1999 and 2000. Hence, in 1997 coccolithophorids were supplying food

to higher trophic levels, perhaps via copepod grazing on microzooplankton (Olson and Strom, 2002). However, the 22:6ω3 was not observed in zooplankton during other years, despite the presence of coccolithophorids during all years studied. Chaetognaths and fish contained substantial amounts of cholesta5,22-dien-3β-ol, cholesterol, and 24-methylcholesta5,22-dien-3β-ol in April of 1998. During other years, the zooplankton contained only trace amounts of sterols. Data from equatorial Atlantic euphausiids (Yuneva *et al.*, 1993) showed that they contained a higher relative abundance of cholesterol than those from higher latitudes. During 1998 the water column had the highest heat content ever recorded (Stabeno *et al.*, 1999), perhaps causing the zooplankton to have lipid compositions resembling those of lower latitude organisms.

Qualitatively, the results of this research are consistent with the ecosystem analysis and carbon budgets for the southeastern Bering Sea developed based on the results of the PROBES study (Walsh and McRoy, 1986). Because spring blooms over the middle shelf were less grazed than those over the outer shelf, Walsh and McRoy (1986) estimated the supply of carbon to the particulate detritus pool at about 190 g C $m^{-2} y^{-1}$ over the middle shelf, almost twice the estimate of 120 g C $m^{-2} y^{-1}$ for the outer shelf. However, their carbon budget neglected the microbial loop, which recent work has shown to be important (Olson and Strom, 2002), and so probably substantially overestimates net production of detrital particulate organic carbon. For comparison, the amount of material collected by the sediment traps from April to October at M2 was 30 g C $m^{-2} y^{-1}$ in 1997, 200 g C $m^{-2} y^{-1}$ in 1998 and 50 g C $m^{-2} y^{-1}$ in 1999. At M3, the quantity collected was 4 g C $m^{-2} y^{-1}$ in 1998 and 30 g C $m^{-2} y^{-1}$ in 1999. The

quantity collected at M3 was less than that at M2, as predicted by Walsh and McRoy (1986) based on greater zooplankton grazing over the outer than the middle shelf. The quantity of organic carbon collected at both M2 and M3 was, on average, much less than they predicted, which is explainable if much of the detrital pool is actually mineralized via the microbial loop in the water column.

The major observations and conclusions of this dissertation research are:

- Fatty acids indicative of diatoms had higher concentrations in M2 than M3 samples. The quantity of diatoms collected by the M2 trap was greater than that for the M3 trap, regardless of variations in weather. The organic matter collected by the M3 trap had more lipids indicative of zooplankton sources than did the M2 trap. Cholesterol was the major neutral lipid deposited to sediment traps at both M2 and M3. It most likely was supplied via zooplankton fecal matter. Together, these observations confirm that grazing of primary production is more extensive over the outer than the middle shelf, although grazing occurred over the middle shelf at most times of year.
- Weather affected the quantity and quality of organic material descending into sediment traps. During cold years, more diatoms and diatom derived lipids were collected in springtime over the middle shelf. During warm years fewer diatoms and more lipids derived from zooplankton were collected during spring. This is consistent with greater coupling between primary and secondary production during warm years.

- Weather affects the timing of spring blooms and the onset of fall mixing, both of which were associated with a marked increase in the quantity of organic matter collected by the sediment traps.
- Substantial quantities of sinking organic matter were collected, at least during some years, as early as March and as late as October. The duration of the productive season in the Bering Sea is longer than earlier research had indicated.
- During 1997-2000 much of production occurred at times other than the April-May spring bloom period. Hence, that time interval is probably not the only one important in controlling the productivity at higher trophic levels in the southeastern Bering Sea.

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Appendix 1. Lipid data tables

Table 1. Sediment trap lipid and C_{org} content.

Fatty acids M2 1997 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid** mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (4/22-5/6)	87.3	281.9	23.5	24.6	1.7	0.09	7.4
2 (5/6-5/20)	24.6	94.1	7.8	2.4	0.17	-	2.2
3 (5/20-5/27)	20.1	41.7	3.5	0.86	0.06	-	1.7
4 (5/27-6/3)*	-	36.2	3	-	-	-	-
5 (6/3-6/10)	44.4	74.3	6.2	3.3	0.23	0.6	3.8
6 (6/10-6/17)	100.4	42.6	3.6	4.3	0.3	0.07	8.5
7 (6/17-6/24)	92	38.7	3.2	3.5	0.25	0.12	7.8
8 (6/24-7/1)	42.8	85.0	7.1	3.6	0.26	0.2	3.7
9 (7/1-7/15)	215.2	229.9	19.2	49.6	3.5	0.7	18.3
10 (7/15-7/29)	38.9	60.3	5.0	2.3	0.16	0.12	3.3
11 (7/29-8/12)	232.3	39.6	3.3	9.1	0.64	0.6	19.5

* Sediment trap sample 4 accidentally pyrolyzed during Soxhlet lipid extraction. When the neutral lipid concentration in sediment trap samples is essentially 0, the neutral lipid:fatty acid ratio data is left blank.
** The mmol lipid-C m^{-2} day^{-1} was calculated after Wakeham *et al.* (1997), assuming that on average, lipids are 85% carbon.

Fatty acids M2 1997 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (9/22-9/29)	41.2	245.3	20.4	10.1	0.72	0.4	3.5
2 (9/29-10/13)	40.6	386.6	32.2	40.8	2.9	0.6	9
3 (10/13-10/27)	205.6	659.3	54.9	135.5	9.6	0.4	17.5
4*							
5*							
6*							
7*							
8**							
9**							
10**							
11**							

*In samples 4-7, the organic carbon content was not quantified during isotopic analysis; therefore, the mg of lipid per g organic carbon could not be calculated. The relative abundance of fatty acids and neutral lipids is reported in tables 2 and 3. **Lipid content in samples 8-11 was too low to be detected.

Fatty acids M2 1998 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (2/26-3/19)	167.7	1207.6	100.6	202.6	14.4	0.8	14.3
2 (3/19-4/2)	69.7	3044.1	253.7	212.1	15	2.4	5.9
3 (4/2-4/16)	9.8	3322.7	276.9	32.8	2.3	0.5	0.8
4 (4/16-4/30)	27.7	2099.6	175	58.3	4.1	0.9	2.3
5 (4/30-5/21)	61.4	1968.2	164	119.1	8.4	1.1	5.1
6 (5/21-6/11)	26.9	447.6	37.3	11.6	0.8	0.2	2.2
7 (6/11-7/2)	61.9	832.6	69.4	51.3	3.6	-	5.2
8 (7/2-7/16)	15.9	564.4	47	9.2	0.7	-	1.4
9 (7/16-7/30)	63.7	515.1	43	33.3	2.4	0.4	5.6
10 (7/30-8/13)	145.3	531.3	44.3	77.1	5.4	0.4	12.3
11 (8/13-9/2)	294.4	1387.7	115.6	408.5	28.9	0.3	25

Fatty acids M2 1998 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (10/10-10/17)*	51	484.8	40.4	24.7	1.7	-	4.3
2 (10/17-10/24)*	16	125.3	10.4	2	0.14	-	1.4
3 (10/24-11/7)*	1.7	215.3	17.9	0.375	0.03	-	0.1
4 (11/7-11/21)*	4	933.9	77.8	3.7	0.26	-	0.3
5							
6							
7							
8							
9							
10							
11							

*Only trace amounts of neutral lipids were found in samples 1-4 during fall 1998. Sediment trap samples after late November were mostly resuspended sediment that contained many phytoplankton fragments.

Fatty acids M2 1999 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (2/10-3/12)	28.6	432.1	36	15.3	1.1	0.2	3.0
2 (3/12-3/26)	41.1	516.5	43	21.3	1.5	0.4	3.5
3 (3/26-4/9)	10.5	427.9	35.7	4.45	0.3	-	0.9
4 (4/9-4/23)	47.5	771.1	64.3	36.5	2.6	0.9	4.0
5 (4/23-5/7)	28.7	421.9	35.2	12.1	0.86		2.4
6 (5/7-5/28)		221.7	18.5				
7 (5/28-6/18)	31.5	516.0	43	16.3	1.2	0.04	2.7
8 (6/18-7/9)	26.3	137.7	11.5	3.6	0.26	0.003	2.2
9 (7/9-7/30)	100.5	162.8	13.6	16.4	1.2	0.4	8.8
10 (7/30-8/20)	162.4	154.9	12.9	25.1	1.8	0.6	14
11 (8/20-9/19	264.6	113.6	9.5	30.1	2.1	0.6	22.1

Fatty acids M2 1999 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (9/26-10/1)	14.3	279.2	23.3	4	0.28	-	1.2
2 (10/1-10/8)	6.6	130.2	10.9	0.9	0.061	-	0.6
3 (10/8-10/22)	80.1	557	46.4	44.5	3.2	1	6.8
4 (10/22-11/5)	84	150.4	12.5	12.6	0.9	0.6	7.2
5 (11/5-11/19)	6.9	149.2	12.4	1.0	0.71	-	0.6
6 (11/19-12/3)	51.7	249.5	20.8	12.8	0.9	0.6	4.4
7 (12/3-12/17)	34.7	362.4	30.2	12.6	0.9	0.6	3.0
8 (12/17-12/31)	112.5	326.3	27.2	36.7	2.6	0.6	9.6
9 (12/31-1/14)	48.1	238.9	20	11.5	0.8	0.3	4.1
10 (1/14-1/28)	211.8	38.9	3.2	8.3	0.6	0.4	18.7
11 (1/28-2/11)	0.812	54.6	4.6	0.13	.009	-	0.2

Fatty acids M2 2000 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (4/25-5/7)	90.7	184.5	15.4	16.7	1.2	3.6	7.8
2 (5/7-5/19)	32.7	163.4	13.6	5.3	0.4	1.3	3
3 (5/19-5/31)	59.7	71.7	6	4.3	0.3	2.1	5
4 (5/31-6/12)	65.5	153.7	12.8	10.1	0.7	1.5	5.5
5 (6/12-6/24)	64.6	101.5	8.5	6.6	0.5	1.5	5.9
6 (6/24-7/7)*	-	-	-	-	-	-	-
7 (7/7-7/20)	171.8	282.4	23.5	48.5	3.4	2.8	14.5
8 (7/20-8/1)	64.6	232.5	19.4	15	1.1	4.2	5.7
9 (8/1-8/14)	102.6	447.2	37.3	45.9	3.3	0.7	8.8
10 (8/14-8/27)	206.2	270.9	22.6	55.8	4	0.6	17.7
11(8/27-9/22)	355.2	430.1	35.8	152.1	10.8	1.1	30.2

*The carbon content of sample 6 was not analyzed.

Fatty acids M2 2000 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (9/22-9/29)	109.5	141.6	11.8	15.5	1.1	0.6	9.3
2 (9/29-10/13)	55.6	79.4	6.6	4.4	0.31	1.7	4.7
3 (10/13-10/27)	73	62.7	5.2	4.6	0.32	1.6	6.2
4 (10/27-11/10)	100.7	77.5	6.4	7.8	0.55	0.6	8.6
5 (11/10-11/24)	77.5	86.4	7.2	6.7	0.5	0.5	6.9
6 (11/24-12/8)	82.1	79.8	6.6	6.5	0.46	0.5	7
7 (12/8-12/22)	103.9	127	10.6	13.2	0.93	0.4	7.1
8 (12/22-1/5)	55	238.7	19.8	13.1	0.93	0.33	4.7
9 (1/5-1/19)	356.3	68.3	5.7	24.3	1.7	0.35	30.2
10 (1/19-2/2)	169	40.3	3.3	6.8	0.48	0.83	14.5
11 (2/2-2/16)*	-	-	-	-	-	-	-

*Sample 11 did not contain enough organic material for analysis.

Fatty acids M3 1998 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (2/26-3/19)	85.6	5.5	0.5	0.5	0.04	-	7.1
2 (3/19-4/2)	64.5	38.7	3.2	2.4	0.17	-	5.3
3 (4/2-4/16)	49.6	63.2	5.3	3.1	0.22	-	4.1
4 (4/16-4/30)	19.6	92.6	7.7	1.8	0.13	-	1.7
5 (4/30-5/21)	53.5	62.7	5.2	3.4	0.24	-	4.6
6 (5/21-6/11)	32.8	5.4	0.45	0.2	0.14	-	3.1

*Samples obtained after 6/11 contained only unidentified fecal pellets. Any lipids were below the detection limit.

Fatty acids M3 1999 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (2/10-3/12)	87.5	80.2	6.7	7	0.5	0.4	7.5
2 (3/12-3/26)	11.2	147.8	12.3	1.7	0.1	0.05	1
3 (3/26-4/9)	13.5	34.9	2.9	0.5	0.03	-	1
4 (4/9-4/23)	3.4	47.7	4	0.2	0.01	-	0.3
5 (4/23-5/7)	52.4	67.9	5.7	3.6	0.3	0.06	5.3
6 (5/7-5/28)	380	172.1	14.3	65.4	4.6	0.5	32.4
7 (5/28-6/18)	197.8	94.7	8	18.7	1.3	0.2	16.5
8 (6/18-7/9)	104.3	85.2	7	8.9	0.6	0.3	9
9 (7/9-7/30)	433	318.5	26.5	137.6	9.7	0.7	36.6
10 (7/30-8/20)	289.3	135.2	11.3	39.1	2.8	0.6	24.8
11 (8/20-9/19	166.7	122.3	10.2	20.4	1.4	0.3	13.7

Fatty acids M3 1999 Sample #	Lipid mg lipid g^{-1} C_{org}	C_{org} mg C m^{-2} day^{-1}	C_{org} mmol C m^{-2} day^{-1}	Lipid mg lipid m^{-2} day^{-1}	Lipid mmol lipid-C m^{-2} day^{-1}	Neutrals / Fatty Acids	Lipid as % of C_{org}
1 (9/26-10/1)	87	1470	122.5	127.6	9	0.8	7.3
2 (10/1-10/8)	186.7	115.5	9.6	21.8	1.5	4	15.6
3 (10/8-10/22)	342.1	160.2	13.5	54.5	3.9	1.1	28.9
4 (10/22-11/5)	406	133.8	11.2	54.2	3.8	2.3	33.9
5 (11/5-11/19)	22.4	78.2	6.5	1.8	0.1	1.1	1.9
6 (11/19-12/3)*	-	-	-	-	-	-	-
7 (12/3-12/17)	222.5	133.6	11.1	29.8	2.1	1.4	19
8 (12/17-12/31)	154.6	85.7	7.1	13.3	0.9	1.8	12.7
9 (12/31-1/14)	131.4	153.4	12.8	23.6	1.7	1.2	13.3
10 (1/14-1/28)	115.6	107.1	8.9	12.4	0.9	1.3	10.1
11 (1/28-2/11)	2.8	50.9	4.2	0.14	0.01	-	0.2

*Sample six was not analyzed.

Table 2. Seasonally averaged relative abundances of fatty acids in sediment trap samples.

M2	Summer 1997	Winter 1997	Summer 1998	Winter 1998
14:0	9.5	25.8	19.1	15.1
15:0	.2		0.5	
15:0 br				
16:0	18.3	19.7	21.3	31.7
16:0br	0.2		0.3	
16:1ω7	20	7.5	10.5	25.8
16:1ω9				
16:2ω6	0.1			
16:3ω3	0.3			
16:4ω3	0.4		0.1	
17:0	2.2	0.1	0.2	
18:0br				
18:0	18.2	15.9	15.3	7.4
18:1ω7	5	2.7	3.4	0.9
18:1ω9	4.9	3.7	3.5	3.2
18:2ω6		0.3	0.1	
18:3ω3	0.4			
18:3ω6				
18:4ω3	0.8	0.5	0.7	
20:0				
20:1ω9	1	1.8	1.7	3.7
20:1ω11	0.9	7	5.9	4.5
20:3	.1	0.1	0.5	
20:4ω6	3.8	2.6	4.4	0.5
20:5ω3	11	6.3	9.3	4.6
22:0				
22:1ω9	0.4	2.9	1.2	1.7
22:3				
22:4	0.4	0.3	0.3	
22:5	1.2	1.7	1.1	0.5
22:6ω3	0.7	1.1	0.6	0.4
TOTAL				
Saturated FA	48.6	61.5	56.7	54.2
Monounsat	32.2	25.6	26.2	39.8
Polyunsat	19.2	12.9	17.1	6

M2	Summer 1999	Winter 1999	Summer 2000	Winter 2000
14:0	10.2	7.5	5.5	6.9
15:0	0.2			0.8
15:0 br				
16:0	15	18.8	22.1	18.1
16:0br			0.2	
16:1ω7	16.9	15.8	17.6	14.4
16:1ω9				0.2
16:2ω6		0.12		
16:3ω3				
16:4ω3				
17:0			0.2	
18:0br				
18:0	13	13.4	12.7	8.6
18:1ω7	3.3	4.8	5.5	3.9
18:1ω9	5.7	4.4	1.4	3.8
18:2ω6				1.6
18:3ω3	0.64	0.3	0.2	
18:3ω6				
18:4ω3	1.7	0.2	0.2	0.9
20:0			0.04	
20:1ω9	7	3.6	7.6	7.7
20:1ω11	0.32	4.5	8.2	2.4
20:3	0.1	1.3	0.6	0.1
20:4ω6	4.6	4.0	1.0	4.4
20:5ω3	14.0	18.1	6.5	12.6
22:0				
22:1ω9	2.7		8.0	4.8
22:3	0.3		0.23	
22:4				1.5
22:5	2.3	1.9	1.3	4.3
22:6ω3	2.1	1.4	1.0	3.2
TOTAL				
Saturated FA	38.4	39.7	40.7	34.4
Monounsat	35.9	33.1	48.3	37
Polyunsat	25.7	27.3	11	28.6

M3	Summer 1998	Summer1999	Winter 1999
14:0	25.1	10.6	2.5
15:0		0.3	
15:0 br			
16:0	27.1	23.5	10.7
16:0br		0.9	
16:1ω7	2.4	6.4	3.2
16:1ω9		0.3	
16:2ω6			0.12
16:3ω3			
16:4ω3			
17:0		0.2	
18:0br			
18:0	15.0	20.0	13.3
18:1ω7	1.0	4.3	4.0
18:1ω9	3.0	2.6	2.2
18:2ω6			
18:3ω3		0.3	0.08
18:3ω6			
18:4ω3		0.7	0.3
20:0			
20:1ω9		5.2	10.6
20:1ω11	1.5	3.7	7.6
20:3		0.4	0.13
20:4ω6	9.0	3.2	4.6
20:5ω3	16.0	10.2	19.1
22:0			
22:1ω9		2.7	10.7
22:3		0.7	1.7
22:4			
22:5	0.5	2.1	5.4
22:6ω3		1.8	3.8
TOTAL			
Saturated FA	67.2	55.5	26.5
Monounsat	7.9	25.2	38.3
Polyunsat	25.5	19.4	35.2

Table 3. Relative abundances of fatty acids during each sediment trap sampling period. Sampling dates are shown in Table 1.

M2 1997 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	10.8	4.0	8.2		6.2	9.9	8.6	10.5	11.3	14.6	10.9
15:0	0.5								1.5		
15:0 br											
16:0	15.2	33.0	27.2		15.3	18.2	20.5	21.9	9.8	16.8	7.5
16:0br									1.6		
16:1ω7	36.2	35.0	27.2		14.2	21.1	22.2	13.6	5	11.6	13.5
16:1ω9											
16:2ω6	1.0										
16:3ω3	2.5										
16:4ω3	3.8										
17:0	0.5				5.2		3.9		1.5	10.5	
18:0br											
18:0	8.8	28.0	14		39	13.8	17.2	14.5	16.7	20.5	10.4
18:1ω7	1.5		8		4.6	6.8	4.8	5	3.5	8.6	6.4
18:1ω9	1.5		8		5	6.3	4.5	6.1	2.6	8.7	6.1
18:2ω6											
18:3ω3	0.5								1.5		2.2
18:3ω6											
18:4ω3	4.8								1.5		2.1
20:0											
20:1ω7	0.4						1.6	2.1	1.4		1.7
20:1ω9	0.4						1.7	4.6	2		3.3
20:3	0.1								1.2		
20:4ω6	2.0				4.2	13.2	2.6	3.3	5.8		6.6
20:5ω3	8.7	0.2	6.4		6.3	10.7	12.4	18.9	19.7	8.5	17.8
22:0											
22:1ω9									1.1		2.6
22:3											
22:4	0.2								1.5		2.1
22:5	0.5								6.8		4.5
22:6ω3	0.4								4		2.3
TOTAL											
Saturated FA	35.3	65	49.4		65.7	41.9	50.2	46.9	42.4	62.4	28.8
Monounsat	39.9	35	43.4		23.8	34.2	34.8	30.9	15.6	28.9	33.6
Polyunsat	24.5	0.2	6.4		10.5	23.9	15	22.2	42	8.5	37.6

M2 1997 Winter	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	15.4	36.1	13.5		11.4	19.1	59.4				
15:0											
15:0 br											
16:0	44.3	13.1	9.1		8.7	21.3	22				
16:0br											
16:1ω7		6.1	6.9		11.3	11.4	9.6				
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0			0.6								
18:0br											
18:0	40.3	15.5	12.1		5.4	13.1	9				
18:1ω7		3.1	3.3		4.6	5.4					
18:1ω9		4.6	5.4		7.4	4.8					
18:2ω6											
18:3ω3			1		1						
18:3ω6											
18:4ω3			1.8		1						
20:0											
20:1ω7			1.3		1	8.5					
20:1ω9		4.1	9.8		15	13.4					
20:3			0.8								
20:4ω6		4.5	6.2		4.9						
20:5ω3		10.5	12.1		11.8	1					
22:0											
22:1ω9			5.8		9.4	2					
22:3											
22:4			1.3		0.8						
22:5		2.1	4.6		3.3						
22:6ω3			4.3		2.6						
TOTAL											
Saturated FA	100	64.7	35.3		25.5	53.5	90.4				
Monounsat		17.9	32.6		48.7	45.5	9.6				
Polyunsat		17.1	32.1		25.4	1					

M2 1998 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	22.3	43.5	18.7	17.1	34	13.4	15	7.9	11.3	10	17
15:0	1				2.4		1.7				
15:0 br											
16:0	6.9	14.1	31	20.8	21.1	46.9	26	20.6	17.8	14.7	14.5
16:0br	1.1				1.7		1				
16:1ω7	4.8	3.6	8.3	13.4	8.5	15.1	15.5	14.1	13.2	7.7	11
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3							1.3				
17:0	0.6				1.3						
18:0br											
18:0	14.7	16.5	18	16.2	14.2	24.6	13.8	16.8	13.6	10.8	9
18:1ω7	2.6	2.3		5.5	1.7		3.4	5.6	4.4	6.8	5
18:1ω9	3.1	2.5		4.9	1.7		3.7	7.9	7.6	4.2	3.2
18:2ω6	0.6										0.8
18:3ω3											
18:3ω6											
18:4ω3	0.8						2		3.7		1.7
20:0											
20:1ω9	1.2	3	5.5	5.8	1.8						1.5
20:1ω7	4.5	2.8			1.6		5.8	12.7	9.4	19.2	8.7
20:3	1.2										4.5
20:4ω6	8.3	3.7	6.7	6.3	3.2		2.3	4.5	4.9	5	2.3
20:5ω3	13.5	8	11.8	10	6.8		7.1	9.9	14.1	9.3	11.2
22:0											
22:1ω9	3.3						1.3			6.2	2
22:3											
22:4	1.9										0.6
22:5	5.4									2	3.5
22:6ω3	2									1	3.1
TOTAL											
Saturated FA	46.6	74.1	67.7	54.1	74.7	84.9	57.5	45.3	42.7	35.5	40.5
Monounsat	19.7	14.2	13.8	29.6	15.3	15.1	29.8	40.3	34.6	47.1	31.4
Polyunsat	33.7	11.7	18.5	16.3	10		12.7	14.4	22.7	17.3	27.7

M2 1998 Winter	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	4.4	15.3	37.3	18	13.8		2				
15:0											
15:0 br											
16:0	10.4	28.5	46.8	23.7	43		38				
16:0br											
16:1ω7	8.9	8.5	13.9	20.4	43.2		60				
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0	7.3	23.8	2	11.6							
18:1ω7	2.7			3							
18:1ω9	6.5	6.7		6.3							
18:2ω6											
18:3ω3											
18:3ω6											
18:4ω3											
20:0											
20:1ω7	8										
20:1ω9	22	7.7		11.2							
20:3											
20:4ω6	2.9										
20:5ω3	12.3	9.5		5.8							
22:0											
22:1ω9	10										
22:3											
22:4											
22:5	2.6										
22:6ω3	2										
TOTAL											
Saturated FA	22.1	67.6	86.1	53.3	56.8		40				
Monounsat	58.1	22.9	13.9	40.9	43.2		60				
Polyunsat	19.8	9.5		5.8							

M2 1999 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	4.3	6.5	8.3	9.1	8.4		13.5	18.4	12.1	13.6	7.7
15:0											
15:0 br							2				
16:0	11	12.4	17.4	14.7	13.8		13	27.7	15.7	15	8.2
16:0br											
16:1ω7	11.4	15.7	26.3	11.1	15		28	28.6	12.5	12	7.6
16:1ω5											
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0	16.7	13.9	16	30.2	21		3.7	8.1	6.5	6.9	6.5
18:1ω7	4.9	4.2	4.3	2.1	2.2		2.8	4.1	2.6	2.5	2.9
18:1ω9	7.7	5.8	5.4	2.8	2.9		4.2	5.9	9.1	7.4	6.1
18:2ω6											
18:3ω3							2.8		1.5	1	1.2
18:3ω6											
18:4ω3							1.7		5.3	6.7	3.9
20:0											
20:1ω7	1.5	1									0.7
20:1ω9	12	9.1	10	3.6	3.4		10.3		5.5	6.1	9.4
20:3											0.8
20:4ω6	5.6	7.4	3.1	7.8	9		3		2.3	2.1	5.5
20:5ω3	11.4	14.6	9.2	17	22.1		7.8	7.2	15.3	15.5	20.6
22:0											
22:1ω11	7.1	3.9					6		2.4	2.5	5.4
22:3		0.7									1
22:4	0.8										
22:5	3.7	3.5		1.6	2.2		0.6		2	2.2	7.1
22:6ω3	1.8	1.3					0.6		6.2	6.5	5.4
TOTAL											
Saturated FA	32	32.8	41.7	54	43.2		32.2	54.2	34.3	35.5	22.4
Monounsat	44.6	39.7	46	19.6	23.5		51.3	38.6	33.1	30.5	32.1
Polyunsat	23.3	27.5	12.3	26.4	33.3		16.5	7.2	32.6	34	45.5

M2 1999 Winter	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0			5.6	7.6		13.4	14.6	13.5	9.8	10.3	
15:0											
15:0 br											
16:0	23.3	49	8	7.6	28.6	17	18.8	12.9	12.3	8.3	
16:0br											
16:1ω7			5.2	6.4	18.4	25.8	25.4	28.3	21.9	26.5	
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0	13	51	9	6.7	22.6	10	7.6	2.9	6.7	3.7	
18:1ω7			2.1			4.3	5	4.6	4.9	6.6	
18:1ω9	17.2		2.9	3.1		8.7	10.6	9.1	8.3	12.7	
18:2ω6								1.6			
18:3ω3											
18:3ω6											
18:4ω3								1.5	1.2		
20:0											
20:1ω7											
20:1ω9	18			3.1	10.8	8.5	14.9	6.5	11	17.7	
20:3						0.2					
20:4ω6			12.2	12.6		2.4		4.4	5	2.9	
20:5ω3	28.5		40.3	39.3	19.6	9.7	3.1	11.9	16.2	11.3	
22:0											
22:1ω9											
22:3											
22:4											
22:5			8	7.6				1.2	1.6		
22:6ω3			6.6	6				1.6	1.1		
TOTAL											
Saturated FA	36.3	100	22.6	21.9	51.2	40.4	41	29.3	28.8	22.3	
Monounsat	35.2		10.2	12.6	18.4	47.3	55.9	48.5	46.1	63.5	
Polyunsat	28.5		67.1	65.5	30.4	12.3	3.1	22.2	25.1	14.2	

M2 2000 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0				13.5	12.1	6.6	6.6	7.1	5.1	4.7	5.8
15:0											
15:0 br											
16:0	17.8	31	39.8	31	30.1	17.4	18.1	27.7	14	9.4	11.7
16:0br									0.6		
16:1ω7	10.4	21.1	36.5	42.8	24.2	12	11.5	17	8.1	7.1	7
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0									1	1.3	0.3
18:0br											
18:0	22.9	15	11.5	4.4	15	11.5	15	13.5	13.8	7.2	12.3
18:1ω7	-	8.7	-	3.8	9.3	6.2	6	8.7	8.2	5.4	5.3
18:1ω9						3.7	3.1		2.8	3.3	3
18:2ω6											
18:3ω3									0.5	1	0.7
18:3ω6											
18:4ω3						0.5			0.7	-	1.2
20:1ω7									0.5	0.7	0.5
20:1ω9	24.7	6.1	4.7	1.7	4.4	21	20.2	15.1	20.2	19.6	17.3
20:1ω11							0.8				
20:3										1	0.8
20:4ω6							0.7		1.1	2.6	2.2
20:5ω3	9.9	7.9	7.5	2.8	4.9	4	4.1	3.5	5.5	12.4	11.3
22:0											
22:1ω9	14.3	10.2				15.1	12.5	7.4	15	12.7	10.4
22:3											
22:4									0.5	1.1	1
22:5						1.2	0.8		2.3	5.2	4.5
22:6ω3						0.8	0.6		0.7	5.3	4.2
TOTAL											
Saturated FA	40.7	46	51.3	48.9	57.2	35.5	39.7	48.3	33.9	22.6	30.6
Monounsat	49.4	46.1	41.2	48.3	37.9	58	54.1	48.2	54.8	48.8	43.5
Polyunsat	9.9	7.9	7.5	2.8	4.9	6.5	6.2	3.5	11.3	28.6	25.9

M2 2000 Winter	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	8.9	8.2	9.9	8.3	7.7	6.9	5.2	5.2	4	4.8	
15:0	0.8		1	1	1	1	0.9	0.8	0.5	0.6	
15:0 br											
16:0	13.3	23.9	25.4	22.3	19.2	19.2	16.3	13.8	13	14.5	
16:0br											
16:1ω7	11.4	16	16.5	16.2	17.5	15.2	13.5	13.2	9.7	14.4	
16:1ω9					1.3	1					
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0	7.3	11.3	7.6	9.4	8.6	6.4	7.7	5.8	12.3	9.6	
18:1ω7	2	4.3	6.5	4.2	3.6	2.4	2.2	3.4	5.6	4.4	
18:1ω9		3.7	7.4	4.7	3.8	3.2	2.3	3.7	4	5.5	
18:2ω6	2.2	0.9	1.1	1	1.7	1.7	1.6	2.2	1.4	2	
18:3ω3											
18:3ω6											
18:4ω3	0.7		1.2	0.9	1.7	0.9	0.8	0.8	0.4	1.4	
20:0											
20:1ω9	0.9	2.2	4.6	6	8.1	14.7	15.2	10.3	11.7	3.6	
20:1ω7	4.3	1.9	2.8	2.3	2.5	1.9	1.7	2	1.6	2.7	
20:3							0.5		0.5		
20:4ω6	6	5.8	2	2.5	2.5	2.8	3.5	6.3	5.5	7.2	
20:5ω3	23.5	13.6	8.8	10	9.6	8.6	9.6	14.7	10	17.5	
22:0											
22:1ω9	3.9		0.7	3.8	4.7	8.6	10	7	8.4	1	
22:3											
22:4	2	1.7		0.7	3.1		1.3	2	2.3	2.2	
22:5	7.6	4.7	2.6	3.7	0.8	3.2	4	5.4	5	6	
22:6ω3	5.2	1.8	1.9	3	2.9	3.3	3.7	3.4	4.1	2.6	
TOTAL											
Saturated FA	30.3	43.4	43.9	41	36.5	33.5	30.1	25.6	29.8	29.5	
Monounsat	22.5	28.1	38.5	37.2	41.2	47	44.9	39.6	41	31.6	
Polyunsat	47.2	28.5	17.6	21.8	22.3	19.5	25	34.8	29.2	38.9	

M3 S98 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	66	25	13.9	11	12.1						
15:0											
15:0 br											
16:0	44	29	25.6	18.4	20.3						
16:0br											
16:1ω7			7.1		5						
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0		18.8	23	20	14.4						
18:1ω7			2		2.5						
18:1ω9			5.8	5.4	4.1						
18:2ω6											
18:3ω3											
18:3ω6											
18:4ω3											
20:0											
20:1ω9			3.2		4.1						
20:1ω7											
20:3											
20:4ω6		6.6	5.3	20	14						
20:5ω3		19.9	14.1	25.2	21						
22:0											
22:1ω9											
22:3											
22:4											
22:5					2.4						
22:6ω3											
TOTAL											
Saturated FA	100	72.8	62.5	49.4	46.8						
Monounsat			18.1	5.4	15.7						
Polyunsat		26.5	19.4	45.2	37.4						

M3 1999 Summer	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	12.8	10.7	28	76.6	8.7	11.5	12.9	0.7	7.3	5.2	4.6
15:0											
15:0 br											
16:0	18.9	23.1	28.2		16.8	12.5	27	18.2	13	11.1	10.1
16:0br	1.6					1.2		1.1	1.6	2.2	2.3
16:1ω7	5.5	8.6			6.9	5.4	14.3	14.6	5	5	4.1
16:1ω9						0.7	0.8	1.2	0.5		
16:2ω6											
16:3ω3											
16:4ω3											
17:0	0.7					0.4	0.4		0.6		
18:0br											
18:0	30.2	27.8	16.2	23.4	18.5	19.2	12.4	11.4	23	16.5	19.3
18:1ω7	4.3	3.3			5.5	3.4	2.7	2.9	1.5	2.5	2.3
18:1ω9	7.4	3.8			7.4	5	6.5	5.1	3.2	4.5	3.5
18:2ω6	0.5					0.4	0.8	1	0.4		
18:3ω3											
18:3ω6											
18:4ω3	0.8					0.9	1.7	2.5	1.2	0.7	
20:0											
20:1ω9	2	9.8	20		15.5	10.4	1.7	3.7	6	6.3	8.8
20:1ω7	2.2	5					0.4	0.7	1	1	1.5
20:3											
20:4ω6	2.2				3.1	5.7	2.1	3.3	6	4.5	7.4
20:5ω3	15.4	2.6			8.2	15.4	10.2	13	17	18.5	20.3
22:0											
22:1ω9	2	1.5			6.5	4.4	0.6	1	3.7	3.3	5.9
22:3											
22:4	3.6				1.2?	0.7	0.6	0.8	1.8	1.2	2.5
22:5	7.3				1.2	1.9	2.3	1.6	5	4.3	5.7
22:6ω3		3.6	7.3			0.6	1.6	2.1	2	1.2	1.7
TOTAL											
Saturated FA		61.6	72.4	100	44	44.8	52.7		45.5		36.3
Monounsat		32	20		41.8	29.3	19.3		20.9		26.1
Polyunsat		6.2	7.3		13.7	25.6	27		33.4		37.6

M3 1999 Winter	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
14:0	4.6		4	3.1			4.2		4	2.8	
15:0											
15:0 br											
16:0	8.7	30.1	5.1	7.1	11.2		8.7	6.2	10.8	8.3	52
16:0br											
16:1ω7	2.8		3.3	2.7			5.2	3.0	7.4	4.2	
16:1ω9											
16:2ω6											
16:3ω3											
16:4ω3											
17:0											
18:0br											
18:0	10	33.2	4.5	13.2	23.2		8	7.5	13.7	6	8
18:1ω7	2.4		2.3	1.2			3	2	5	4.3	
18:1ω9	4.5		4.6	3.5			4.6	3	8	7	
18:2ω6	0.7										
18:3ω3											
18:3ω6											
18:4ω3	1.4		1.3								
20:0											
20:1ω9	10.2	22.2	9	3.7	11.5		15.6	22	1.7		
20:1ω7	2.1	3.4	1.7	7.8				11	22.3	20.1	
20:3											
20:4ω6	4.7		6.8	11.5			5.9	7		6	
20:5ω3	20.4		29	24.4	37.5		20	17.3	9.7	14.3	
22:0											
22:1ω9	8.3	10.6	5.9	6.1	11.9		13	10	16	15.4	
22:3											
22:4	2		2.6	4			2	2.2	0.4	2.1	
22:5	7		8.5	8.2	4.5		6.6	6.3	0.7	5.3	
22:6ω3	11.2		11.6	3.6			3.2	2.6			
TOTAL											
Saturated FA	23.3	63.3	13.6	23.4	34.4		20.9	13.7	28.5	17.1	
Monounsat	30.3	36.2	26.8	25	23.4		41.4	51	60.4	51	
Polyunsat	47.4		59.8	51.7	42		37.7	35.4	10.8	27.7	

Table 4. Seasonally averaged relative abundances of neutral lipids.

Mooring site M2	Summer 1997	Winter 1997	Summer 1998	Winter 1998
16:0 OH				
18:0 OH	0.1	0.1	0.3	
Phytol	3		0.2	
20:1 OH	3.1	12.8	9.3	
22:1 OH	0.1	2	8.2	
24-norcholesta-5.22E-dien-3β-ol	0.5	0.9	2.1	
5α24-norcholest-22E-en-3β-ol				
27-nor-24-methylcholesta-5,22E-dien-3β-ol	1.1	4.6	0.8	
Cholesta-5,22E-dien-3β-ol	0.8	1.1	5.5	
Cholesta-5,24(28)-dien-3β-ol	0.1	0.4	0.6	
5α-cholest-22E-en-3β-ol				
Cholest-5-en-3β-ol	88	71.8	66.3	
5α-cholestan-3β-ol			0.2	
24-methylcholesta-5,22E-dien-3β-ol	0.2	0.4	0.8	
24-methyl-5α-cholest-22E-en-3β-ol				
24-methylcholesta-5,24(28)E-dien-3β-ol				
24-methylcholest-5-en-3β-ol				
24-ethylcholesta-5,24E-dien-3β-ol				
24-ethylcholest-5-en-3β-ol				
4α,23,24-trimethyl-5a-cholest-22E-en-3β-ol				
Glyceryl ether 16:0	0.4	2.7	1.4	
Glyceryl ether 18:0	0.4	0.2	0.3	
Glyceryl ether 18:1a	2	2.3	2.3	
Glyceryl ether 18:1b	0.2	0.1	0.7	
Glyceryl ether 20:1		0.6	1	
TOTAL				
Fatty alcohol	6.3	14.9	18	
Sterols	90.7	79.2	76.3	
DAGE	3	5.9	5.7	

Mooring site M2	Summer 1999	Winter 1999	Summer 2000	Winter 2000
14:0 OH	0.9			
16:0 OH	6.4		0.7	2.6
18:0 OH	0.8		0.7	6.8
18:1 OH	0.4	0.9		
Phytol			4.6	3.5
20:1 OH	17.1	66.6	14.3	6.7
22:1 OH	0.9		17.7	9.3
24-norcholesta-5.22E-dien-3β-ol	3	2.2	2.3	4.4
5α24-norcholest-22E-en-3β-ol				
27-nor-24-methylcholesta-5,22E-dien-3β-ol	0.2		3.5	1.7
Cholesta-5,22E-dien-3β-ol	4.3	1.3	3.5	6.1
Cholesta-5,24(28)-dien-3β-ol	0.9		0.1	1.3
5α-cholest-22E-en-3β-ol				
Cholest-5-en-3β-ol	53.6	23.9	47.3	37.8
5α-cholestan-3β-ol	0.2		0.2	0.9
24-methylcholesta-5,22E-dien-3β-ol	1.5	0.6	1.3	5.1
24-methyl-5α-cholest-22E-en-3β-ol			0.7	
24-methylcholesta-5,24(28)E-dien-3β-ol	1.3		0.8	3.4
24-methylcholest-5-en-3β-ol				0.4
24-ethylcholesta-5,24E-dien-3β-ol				0.5
24-ethylcholest-5-en-3β-ol				1.3
4α,23,24-trimethyl-5a-cholest-22E-en-3β-ol				
Glyceryl ether 16:0	3.2	1.1	0.6	1.9
Glyceryl ether 18:0	1.2	3.1	0.1	0.3
Glyceryl ether 18:1a	1.7	0.3	0.7	2.3
Glyceryl ether 18:1b	0.4			0.2
Glyceryl ether 20:1	2		0.9	3.5
TOTAL				
Fatty alcohol	26.5	67.5	38	28.9
Sterols	65	28	59.7	62.9
DAGE	8.5	4.5	2.3	8.2

Mooring Site M3	Summer 1998	Summer 1999	Winter 1999
14:0 OH			
16:0 OH		2.9	
18:0 OH		3.5	0.8
18:1 OH			
Phytol		0.8	
20:1 OH		11.3	4.3
22:1 OH		14.9	9.5
24-norcholesta-5.22E-dien-3β-ol		2.4	4.3
5α24-norcholest-22E-en-3β-ol			
27-nor-24-methylcholesta-5,22E-dien-3β-ol		2.9	6.1
Cholesta-5,22E-dien-3β-ol		2.6	6
Cholesta-5,24(28)-dien-3β-ol		0.3	0.7
5α-cholest-22E-en-3β-ol			
Cholest-5-en-3β-ol		39.6	54.3
5α-cholestan-3β-ol		0.5	1
24-methylcholesta-5,22E-dien-3β-ol		1.4	6.4
24-methyl-5α-cholest-22E-en-3β-ol			0.8
24-methylcholesta-5,24(28)E-dien-3β-ol		0.5	1.5
24-methylcholest-5-en-3β-ol		0.1	0.3
24-ethylcholesta-5,24E-dien-3β-ol			0.6
24-ethylcholest-5-en-3β-ol			0.3
4α,23,24-trimethyl-5a-cholest-22E-en-3β-ol			0.1
Glyceryl ether 16:0		4.6	0.2
Glyceryl ether 18:0		1.7	
Glyceryl ether 18:1a		5.5	0.5
Glyceryl ether 18:1b		2.2	0.4
Glyceryl ether 20:1		2.3	1.9
TOTAL			
Fatty alcohol		33.4	14.6
Sterols		50.3	82.4
DAGE		16.3	3